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(54) Title: THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS

(57) Abstract: An outbreak of a virulent respiratory virus, now known as Severe Acute Respiratory Syndrome (SARS), was identified in Hong Kong, China and a growing number of countries around the world in 2003. The invention relates to nucleic acids and proteins from the SARS coronavirus. These nucleic acids and proteins can be used in the preparation and manufacture of vaccine formulations, diagnostic reagents, kits, etc. The invention also provides methods for treating SARS by administering small molecule antiviral compounds, as well as methods of identifying potent small molecules for the treatment of SARS.

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THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS

All documents cited herein are incorporated by reference in their entirety.

RELATED APPLICATIONS, FROM WHICH PRIORITY IS CLAIMED

This application incorporates by reference in its entirety US provisional patent application 60/462,218, Attorney Reference No. PP20474.001, filed on April 10, 2003 via Express Mail with the US post office, US provisional patent application 60/462,465, Attorney Reference No. PP20480.001, filed on April 11, 2003 via Express Mail with the US post office, US provisional patent application 60/462,418, Attorney Reference No. PP20480.002, filed on April 12, 2003 via Express Mail with the US post office, US provisional patent application 60/462,748, Attorney Reference No. PP20480.003, filed on April 13, 2003 via Express Mail with the US post office, US provisional patent application 60/463,109, Attorney Reference No. PP20480.004, filed on April 14, 2003 via Express Mail with the US post office, US provisional patent application 60/463,460, Attorney Reference No. PP20480.005, filed on April 15, 2003 via Express Mail with the US post office, US provisional patent application 60/463,668, Attorney Reference No. PP20480.006, filed on April 16, 2003 via Express Mail with the US post office, US provisional patent application 60/463,983, Attorney Reference No. PP20480.007, filed on April 17, 2003 via Express Mail with the US post office, US provisional patent application 60/463,971, Attorney Reference No. PP20480.008, filed on April 18, 2003 via Express Mail with the US post office, US provisional patent application 60/464,899, Attorney Reference No. PP20480.009, filed on April 22, 2003 via Express Mail with the US post office, US provisional patent application 60/464,838, Attorney Reference No. PP20507.001, filed on April 22, 2003 via Express Mail with the US post office, US provisional patent application 60/465,273, Attorney Reference No. PP20518.001, filed on April 23, 2003 via Express Mail with the US post office, US provisional patent application 60/465,535, Attorney Reference No. PP20518.002, filed on April 24, 2003 via Express Mail with the US post office, US provisional patent application 60/468,312, Attorney Reference No. PP20480.010, filed on May 5, 2003 via Express Mail with the US post office, and US provisional patent application 60/473,144, Attorney Reference No. PP20480.011, filed on May 22, 2003, US provisional patent application 60/495,024, Attorney Reference No. PP20480.012, filed on August 14, 2003 via Express Mail with the US post office, US provisional patent application 60/505,652, Attorney Reference No. PP20480.013, filed on September 23, 2003 via Express Mail with the US post office, US provisional patent application 60/510,781, Attorney Reference No. PP20480.014, filed on October 11, 2003 via Express Mail with the US

post office, US provisional patent application 60/529,464, Attorney Reference No. PP20480.015, filed on December 11, 2003 via Express Mail with the US post office, US provisional patent application 60/536,177, Attorney Reference No. PP20480.016, filed on January 12, 2004 via Express Mail with the US post office, and US provisional patent application 60/___,___, Attorney Reference No. PP20480.017, filed on April 7, 2004 via Express Mail with the US post office.

FIELD OF THE INVENTION

The invention relates to nucleic acids and proteins from Severe Acute Respiratory Syndrome (SARS) Virus. These nucleic acids and proteins can be used in the preparation and manufacture of vaccine formulations for the treatment or prevention of SARS. The invention also relates to diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention also relates to methods for the treatment or prevention of SARS utilizing small molecule viral inhibitors and combinations of small molecule viral inhibitors and kits for the treament of SARS.

BACKGROUND OF THE INVENTION

An outbreak of a virulent respiratory virus, now known as Severe Acute Respiratory Syndrome (SARS), was identified in Hong Kong, China and a number of other countries around the world in 2003. Patients typically had symptoms including fever, dry cough, dyspnea, headache, and hypoxemia. Isolates of the SARS virus appear to have homology with at least the RNA polymerase gene of several known coronaviruses. A phylogenetic analysis of this homology is presented in Peiris *et al.*, "Coronavirus as a possible cause of severe acute respiratory syndrome", *Lancet*, published online April 8, 2003 at *http://image.thelancet.com/extras/03art3477web.pdf*, incorporated herein by reference in its entirety. Other sequenced fragments of the SARS virus genome appear to overlap with the open reading frame 1b of coronaviruses. See, Drosten *et al.*, "Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome", *New England Journal of Medicine*, published online at *http://www.nejm.org* on April 10, 2003, incorporated herein by reference in its entirety.

The Genome Science Center in British Colombia, Canada published on its website (*http://www.bcgsc.ca/bioinfo/SARS/*) a draft genome assembly of 29,736 base pairs of a virus believed to be a SARS virus, referred to as the TOR2 isolate. This draft genome assembly is given herein as SEQ ID NO: 1.

The Centers for Disease Control (CDC) published a nucleotide sequence of a SARS-CoV strain (SEQ ID NO: 2) on its website (*http://www.cdc.gov/ncidod/sars/pdf/nucleoseq.pdf*). The CDC

has also published a phylogenetic tree of the predicted N, S and M proteins (attached as FIGURE 6). This tree places the SARS virus outside any of the previously known coronavirus groups.

There is a growing need for prophylactic or therapeutic vaccines against the SARS virus as well as diagnostic and screening methods and compositions to identify the presence of the virus in, *e.g.*, mammalian tissue or serum.

SUMMARY OF THE INVENTION

The invention relates to nucleic acids and proteins from Severe Acute Respiratory Syndrome (SARS) virus. These nucleic acids and proteins can be used in the preparation and manufacture of vaccine formulations for the treatment or prevention of SARS. Such vaccine formulations may include an inactivated (or killed) SARS virus, an attenuated SARS virus, a split SARS virus preparation and a recombinant or purified subunit formulation of one or more SARS viral antigens. Expression and delivery of the polynucleotides of the invention may be facilitated via viral vectors and/or viral particles.

The invention also relates to diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention further includes non-coding SARS viral polynucleotide sequences, SARS viral sequences encoding for non-immunogenic proteins, conserved and variant SARS viral polynucleotide sequences for use in such diagnostic compositions and methods.

The invention further relates to vaccine formulations comprising one or more SARS virus antigens and one or more other respiratory virus antigens. Additional respiratory virus antigens suitable for use in the invention include antigens from influenza virus, human rhinovirus (HRV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, and rhinovirus. The additional respiratory virus antigen could also be from a coronavirus other than the SARS coronavirus. Preferably, the additional respiratory virus antigen is an influenza viral antigen.

The compositions of the invention may further comprise one or more adjuvants. Adjuvants suitable for use in the invention include mucosal, transdermal or parenteral adjuvants. Mucosal adjuvants suitable for use in the invention include detoxified bacterial ADP-ribosylating toxins, such as *E. coli* heat labile toxoids (*e.g.*, LTK63), chitosan and derivatives thereof, and non-toxic double mutant forms of *Bordetella pertussis* toxoids. Parenteral adjuvants suitable for use in the invention include MF59 and aluminum or aluminum salts.

The invention also provides methods for treating SARS by administering small molecule compounds, as well as methods of identifying potent small molecules for the treatment of SARS.

In one aspect of the invention a method of identifying a therapeutically active agent is provided comprising: (a) contacting the therapeutically active agent with a cell infected with the SARS virus; (b) measuring attenuation of a SARS related enzyme.

In a more particular embodiment, the therapeutically active agent is a small molecule. In another more particular embodiment, the therapeutically active agent is a nucleoside analog. In another more particular embodiment the therapeutically active agent is a peptoid, oligopeptide, or polypeptide. In another embodiment the SARS related enzyme is SARS protease. In another embodiment the SARS related enzyme is SARS polymerase. In still another embodiment the SARS related enzyme is a kinase. Methods of identifying therapeutically active agents for treatment of SARS virus infection are further discussed in Section V below.

In another aspect of the invention a method of treating a human infected with SARS is provided comprising administering a small molecule to a patient in need thereof. In one embodiment the small molecule is an inhibitor of SARS protease. In another embodiment the small molecule is an inhibitor of SARS polymerase. In another embodiment the SARS related enzyme is a kinase. In still another embodiment the small molecule is administered orally or parenterally.

The invention also provides the use of such small molecules in the manufacture of a medicament for the treatment of severe acute respiratory syndrome.

Small molecule compounds of the present invention include those of less than 1000 g/mol, preferably with an aromatic region and greater than one heteroatom selected from O, S, or N. Preferred small molecules include, but are not limited to acyclovir, gancyclovir, vidarabidine, foscamet, cidofovir, amantidine, ribavirin, trifluorothymidine, zidovudine, didanosine, zalcitabine, and combinations thereof. Interferons may also be used for treating patients, including interferon-α and interferon-β. Interferon treatment has shown promise in treating SARS in monkeys (Enserink (2004) *Science* 303:1273-1275), particularly when pegylated (Haagmans *et al.* (2004) *Nature Medicine* 10:290-293).

One aspect of the present invention relates to methods for identifying individuals exposed to, and biological samples containing SARS virus (SARSV), and to kits for carrying out the methods. Such methods can utilize nucleic acid detection techniques such as PCR, RT-PCR (the *Coronaviridae* are RNA viruses), transcription-mediated amplification (TMA), ligase chain reaction (LCR), branched DNA signal amplification assays, isothermal nucleic acid sequence based amplification (NASBA), other self-sustained sequence replication assays, boomerang DNA amplification, strand-displacement activation, cycling probe technology, or combinations of such amplification methods. Such nucleic acid detection techniques utilize oligonucleotides having nucleotide sequence similar to, or complementary to, the SARS viral genome, as primers (*e.g.*, for amplification) and as probes (*e.g.*, for capture or detection), as is well known in the art.

Alternatively, or in addition to the nucleic acid detection methods described supra, the methods of the present invention can utilize various immunoassay techniques for detection of SARSV antigens and/or antibodies.

Accordingly, the present invention relates to methods of identifying individuals exposed to SARSV, or biological samples containing SARSV, by detecting the presence of SARSV antigens using antibodies which specifically bind to the same. The antibodies are preferably monoclonal antibodies. Quantification of the amount of viral antigens present in a sample of an individual may be used in determining the prognosis of an infected individual. Preferably, the SARSV antigens to be detected are generally one of the structural proteins, particularly those present on the surface of the viral particles and include, for example, the spike glycoprotein (S), also called E2; the envelope (small membrane) protein (E), also called sM; the membrane glycoprotein (M), also called E1; the hemagglutinin-esterase glycoprotein (HE); also called E3; and the nucleocapsid phosphoprotein (N). In preferred embodiments, the antigens to be detected are the S, E and M proteins using antibodies to the same.

The present invention relates to kits for identifying individual SARSV and reagents used in such kits. The kits comprise a first container which contains antibodies which specifically bind to a SARSV antigen and a second container which contains the SARSV antigen. The antibodies are preferably monoclonal antibodies. The kits may be adapted for quantifying the amount of antigen in a sample of an individual. Such information may be used in determining the prognosis of an infected individual.

The present invention relates to methods of identifying individuals exposed to SARS virus, or biological samples containing SARSV, by detecting the presence of antibodies against SARS virus antigen in a sample using SARS antigen. Quantification of the amount of anti-SARS protein from SARS antibodies present in a sample of an individual may be used in determining the prognosis of an infected individual. Any one or more of the viral proteins (structural proteins or nonstructural proteins) may be used as antigen to detect the SARSV antibodies; preferably a SARSV antigen that is conserved amoung SARSV isolates is preferred. In this regard, nonstructural protein (*e.g.*, Pol, Hel, 3CLp, MP, PLP1, PLP2) may be particularly useful.

The present invention relates to kits for identifying individuals exposed to SARS and reagents used therein. The kits comprise a first container which contains antibodies which were produced in response to exposure to an antigen from SARS virus and a second container which contains the SARS antigen(s). The kits may be adapted for quantifying the amount of anti-SARS antibodies present in a sample of an individual. Such information may be used in determining the prognosis of an infected individual.

The present invention relates to methods of identifying individuals exposed to SARS virus, or biological samples containing SARSV, by detecting the presence of nucleic acid from SARS

virus. Quantification of the amount of SARS nucleic acid present in a sample of an individual may be used in determining the prognosis of an infected individual. The methods utilize oligonucleotide probes and/or primers that are similar or complementary in sequence to the SARSV genome or transcription or replication products. Preferred probes and primers are described herein. Also included in the present invention are kits for carrying out the methods of detecting the SARSV nucleic acid.

The invention further includes a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. In another embodiment, the antiviral compound is an inhibitor of an RNA-dependent RNA polymerase. In another embodiment, a first antiviral compound which is a protease inhibitor is administered with a second antiviral compound which is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2.

The invention further provides for a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2 by inhalation. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. In another embodiment, the antiviral compound is an inhibitor of an RNA dependent RNA polymerase. In another embodiment, a first antiviral compound which is a protease inhibitor is administered with a second antiviral compound which is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2 by inhalation. The steroidal anti-inflammatory drug may be administered by inhalation for a local effect or administered for systemic absorption such as via an oral or intravenous route.

The invention further provides the use of an antiviral compound, as defined above, in the manufacture of a medicament for the treatment of severe acute respiratory syndrome.

The invention further provides for a kit for use by a consumer for the treatment and/or prevention of SARS. Such a kit comprises: (a) a pharmaceutical composition comprising a therapeutically effective amount of at least one antiviral compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2 and a pharmaceutically acceptable carrier, vehicle or diluent; (b) a container for holding the pharmaceutical composition; and, optionally; (c) instructions describing a method of using the pharmaceutical compositions for the treatment and or the prevention of SARS. The kit may optionally contain a plurality of antiviral compounds for the treatment of SARS wherein the anti viral compounds are selected from 3C-like protease inhibitors and papain-like protease inhibitors. In a further embodiment, the kit contains an antiviral compound which is an RNA-dependent RNA polymerase inhibitor. When the kit comprises more than one antiviral compound, the antiviral compounds contained in the kit may be optionally combined in the same pharmaceutical composition.

An additional aspect of the invention provides for the use of at least one of the antiviral compounds described in the US Patents and published international patent applications listed in Table 1 and Table 2 for the manufacture of a medicament for the treatment or prevention of SARS.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Schematic of coronavirus genome organization.

FIGURE 2: Schematic of coronavirus ORF1a/ORF1b gene products.

FIGURE 3 (A - C): Alignment of coronavirus polynucleotide sequences for selected genes (including nucleocapsid (N), matrix (M), and hemagluttinin-esterase (HE)).

FIGURE 4 (A - F): Alignment of coronavirus polypeptide sequences (including ORF1a/ORF1b, nucleocapsid (NP), hemagluttinin-esterase (HE), envelope (Sm or E), matrix (M), and spike (S).

FIGURE 5: Alignment of spike (S) polypeptide sequences, taken from Figure 4, in the region of the junction of the S1 and the S2 domains, and protease cleavage site for selected coronaviruses.

FIGURE 6: CDC phylogenetic tree of SARS-CoV strain (Clustalx 1.82, neighbor-joining tree). Figure 6A shows coronavirus N protein analysis, Figure 6B shows coronavirus S protein analysis, and Figure 6C shows coronavirus M protein analysis.

FIGURE 7: Conserved and specific sequence of the SARS virus. Figures 7A-7D show multiple sequence alignments (CLUSTAL W 1.82) of the structural proteins of the SARS virus genome (7A: PEP4 Spike protein; 7B: PEP7 small membrane protein; 7C: PEP8 matrix glycoprotein; 7D: PEP13 nucleocapsid protein), which have counterparts in all or some of the other known coronaviruses. Figures 7E-7H show dendrograms reporting the protein distances among the

sequences in alignments 7A-7D. Labels 229E: human coronavirus; MEV: murine hepatitis virus; TGV: transmissible gastroenteritis virus; AIBV: avian infectious bronchitis virus; BOVINE: Bovine coronavirus; PEDV: porcine epidemic diarrhea virus.

FIGURE 8: Alignment of the 5'UTR of several coronaviruses, to show consensus nucleotide sequence at the 5'UTR.

FIGURE 9: Sequences of preferred primers for amplification of the 5'UTR. F and R denote forward and reverse PCR primers, and the numbers indicate nucleotide positions withing Figure 8.

FIGURE 10: Alignment of the 3'UTR of several coronaviruses, to show consensus nucleotide sequence at the 3'UTR.

FIGURE 11: Sequences of preferred primers for amplification of the 3'UTR. F and R denote forward and reverse PCR primers, and numbers indicate nucleotide positions within Figure 10.

FIGURE 12: Coiled-coil prediction for SEQ ID NO: 6042, using Coils program (Figure 12A) or LearrCoil (Figure 12B).

FIGURE 13: Example of insertion of a reporter gene-of-interest at a site between exisiting SARS virus genes. Small nonstructural gene products are not depicted schematically.

FIGURE 14: Schematic depicting representative examples of SARS virus replicons. Small nonstructural gene products are not depicted schematically.

FIGURE 15: SARS virus nsp2 proteinase (3CLp) and identification of catalytic and substrate sites.

FIGURE 16: alignment of SARS virus nsp2 proteinase (3CLp) with that of avian IBV, MHV, and BCoV. Residues in dotted boxes are key residues the substrate sites (F, Y & H); residues in solid boxes are catalytic cysteine (C) and histidine (H) residues.

FIGURE 17: Genome organization of SARS coronavirus. Replicase and structural regions are shown, along with the predicted products of cleavage within ORF1a and ORF1b. The position of the 5' RNA leader sequence (L), the 3' poly(A) tract and the ribosomal frame-shift consensus between ORF1a and ORF1b are also indicated. Each box represent a protein product. They are shaded according to the level of amino acid identity with corresponding proteins of other coronaviruses (see also Table 2). The SARS-specific genes are white. Positions of the 9 SARS-specific six-base IG sequences (5'-ACGAAC-3'; SEQ ID NO 7293) are indicated by arrows.

FIGURE 18: Genome organization of Coronaviruses representative of group 1 (HCoV-229E, accession number: AF304460), group 2 (mouse hepatitis virus MHV, accession number: NC_001846), group 3 (avian infectious bronchitis virus AIBV, accession number: NC_001451)

and SARS coronavirus. Other completely sequenced coronaviruses used in this study are available at the following accession numbers: porcine epidemic diarrhea virus (PEDV), AF353511; transmissible gastroenteritis virus (TGV), NC_002306; Bovine coronavirus (BCoV): AF220295. Red boxes represent group-specific genes. The position of the leader RNA sequence and poly(A) tract is also indicated in genomes where they are reported. The position of specific IG sequences is indicated by circles of different shades. In the SARS genome, we also find three IG sequences specific for group 2 coronavirus.

FIGURE 19: Topological model predicted for the spike protein anchored to the viral membrane. Structural and predicted functional domains are indicated. The N-terminal region (S1) is predicted to contain the receptor binding domain. Two coiled coil regions within the S2 domain, partially superimposed to leucine zipper motifs are presumably involved in oligomerization. The hydrophobic domain is responsible for membrane anchoring.

FIGURE 20: Phylogenetic tree obtained from the multiple sequence alignment of a 922 bp internal region of the *pol* gene from 12 coronaviruses and SARS. Numbers at the nodes represent the result of a bootstrap analysis and strongly support the branches. Sequences not available within the complete coronavirus genomes have been retrieved from GenBank at the following accession numbers: hemagglutinating encephalomyelitis virus of swine (PHEV), AF124988, Human OC43 virus (OC43), AF124989, canine coronavirus (CCV), AF124986, feline infectious peritonitis virus (FIPV), AF124987, turkey coronavirus (TCV), AF124991, syaloacryoadenitis virus of rats (SDAV), AF124990.

FIGURE 21: 21A. Unrooted tree obtained from the alignment of consensus sequences of the group I and group II S1 domain of spike proteins (G1_cons and G2_cons) with those of a group 3 spike (AIBV) and the spike of SARS virus. The number indicates the result of a bootstrap analysis. The sequences used to generate the consensus profile from group 1 are: HcoV-229E, accession number P15423; porcine epidemic diarrhea virus (PEDV), acc no: NP_598310; transmissible gastroenteritis virus (TGV), acc no: NP_058424; Canine coronavirus (CCV), acc no: S41453; porcine respiratory virus (PRV), acc no: S24284; feline infectious peritonitis virus (FIPV), acc no: VGIH79. The sequences used to generate the consensus profile from group 2 are: mouse hepatitis virus (MHV), acc no: NP_045300; Bovine coronavirus (BCoV), acc no: NP_150077; Human coronavirus OC43, acc no: P36334; hemagglutinating encephalomyelitis virus of swine (PHEV), acc no: AAL80031; for group 3, only the sequence of the spike protein of avian infectious bronchitis virus (AIBV), acc no: AAO34396 was used. 21B: Schematic representation of cysteine positions in S1 domains of group 1, 2 and 3, compared to the SARS spike. Horizontal bars represent the S1 amino acid sequences (in the case of SARS and AIBV) or the consensus profiles (generated from group 1, G1_cons, and from group 2, G2_cons). The

length of the bars are not to scale. Relative cysteine positions are indicated by rectangle bars. Only cysteines perfectly conserved within each consensus are reported. Lines connect cysteines conserved between the SARS S1 domain and the consensus sequences as shown.

FIGURE 22: illustration of a Neisseria Adhesin A protein (NadA).

FIGURE 23: Raw translation from SARS coronavirus genome (reading frame +1).

FIGURE 24: Raw translation from SARS coronavirus genome (reading frame +3)

FIGURE 25: 1b and Spike open reading frames, separated by *.

FIGURE 26: SARS growth in vero cells.

FIGURE 27: chromatogram of the capture step of SARS coronavirus on Matrix Cellufine Sulfate Superformance 150/10. Analysis was on 100ml coronoavirus harvest. The left Y axis shows absorbance at 280nm. The right Y axis shows the gradient (%B). The X axis shows the volume (ml).

FIGURE 28: Silver-stained MCS chromatography fractions. Lanes are: (1) marker; (2) coronavirus vero cell harvest; (3) coronavirus vero cell harvest, after 0.65μm filtration; (4) flowthrough; (5) wash; (6) 20% peak (virus peak). Lanes were loaded with 1 μg of test protein.

FIGURE 29: Western Blot of MCS chromatography fractions. Lanes are as described for Fig.28.

FIGURE 30: Linear density gradient ultracentrifugation, 15-60% sucrose (SW28, 2 hours, 20000 rpm). The graph shows protein concentration (■) and sucrose concentration (♦).

FIGURE 31: Silver-stained density gradient fractions on NuPage 4-12% Bis-Tris-Ge (Novex), reduced conditions, heated for 10 minutes at 70°C. Lanes are: (1) marker; (2) 20% peak MCS; (3) density gradient fraction 11; (4) density gradient fraction 12; (5) density gradient fraction 13; (6) density gradient fraction 14; (7) density gradient fraction 15; (8) density gradient fraction 16; (9) density gradient fraction 17. The bulk of proteins was in fractions 15 to 17. Lanes 2, 8 and 9 were loaded with 1μg protein.

FIGURE 32: Chromatogram of the Capture Step of SARS coronavirus on MCS. Details are as for Figure 27, except that 200ml harvest was used.

FIGURE 33: Silverstain (left) and Western Blot (right) of chromatographic fractions. Lanes are as described for Figures 28 and 29, except that lane (6) is the 5% peak. Treatment before SDS-PAGE was at room temperature for 30 minutes.

FIGURE 34: Density Gradient Ultracentrifugation, 15-40% sucrose (SW28, 2 hours, 20000 rpm). The graph shows protein concentration (■) and sucrose concentration (♦).

FIGURE 35: Silverstain (left) and Western Blot (right) of Density Gradient Ultracentrifugation fractions on NuPage 4-12% Bis-Tris-Ge (Novex), reduced conditions. Lanes are: (1) marker; (2) density gradient fraction 6; (3) density gradient fraction 7; (4) density gradient fraction 8; (5) density gradient fraction 9; (6) density gradient fraction 10; (7) density gradient fraction 15. Fractions 7-10 (lanes 3-6) contained pure coronavirus proteins. The bulk of impurities was in fraction 15 (lane 7). Lanes 2, 8 and 9 were loaded with ~1μg protein. Treatment before SDS-PAGE was at room temperature for 30 minutes.

FIGURE 36: EM pictures of Density Gradient Fractions 8-10. Figure 36A shows fraction 8; Figure 36B shows fraction 9; Figure 36C shows fraction 10.

FIGURE 37: Spike/NadA fusion constructs.

FIGURES 38 and 39: Results of the expression in *E.coli* of $S1_L$, $S1_L$-NadA and $S1_L$-$NadA_{\Delta anchor}$. Figure 38 shows SDS-PAGE analysis of total lysates from BL21(DE3)/pET, BL21(DE3)/pET-$S1_L$ and BL21(DE3)/pET-$S1_L$-$NadA_{\Delta anchor}$. The bands are indicated by an arrow, and the three lanes are, from left to right: BL21(DE3)/pET; BL21(DE3)/pET-$S1_L$; BL21(DE3)/pET-$S1_L$-$NadA_{\Delta anchor}$. Figure 39 shows (39A) SDS-PAGE and (39B) western blot analyses of total lysates from BL21(DE3)/pET, BL21(DE3)/pET-$S1_L$-NadA (grown under un-induced condition) and BL21(DE3)/pET-$S1_L$-NadA (grown under induced condition). The bands are indicated by an arrow, and lanes are, from left to right: BL21(DE3)/pET; BL21(DE3)/pET-$S1_L$-NadA; BL21(DE3)/pET-$S1_L$-NadA. The western blot shows the presence of oligomeric forms of the protein.

FIGURE 40: Schematic of SARS Spike clones.

FIGURE 41: Transient Expression of SARS Spike Proteins (western blot of COS7 cell lysate). Each lane of the 4-20% TG SDS gel was loaded with 20μg cell lysate (total 1.2mg). The labeling antibodies are shown.

FIGURE 42: Western blot analyses of COS7 cell lysates on 4% TG SDS gel showing oligomerization state of intracellular S molecules.

FIGURE 43: Western blot analyses of COS7 cell lysates on 4-20% TG SDS gel showing Transient Expression of SARS Spike Proteins. Lanes are: (1) mock, AF; (2) mock, DF; (3) nSh, AF; (4) nSh, DF; (5) nShΔTC, AF; (6) nShΔTC, DF. Each lane was loaded with 5μl of each sample, 400μl total. The blot was labeled with antibody against the His-tagged protein.

FIGURE 44: Western blot analyses of COS7 cell medium on 4-20% TG SDS gel showing Transient Expression of SARS Spike Proteins. Truncated spike protein is secreted. Spike proteins were purified from the culture medium (from a 10cm plate), first by a ConA column and then finally by His•tag Magnetic beads. Each lane was loaded with one third of the material.

FIGURE 45: Western blot analyses of COS7 cell lysates on 4-20% TG SDS gel showing glycoslation of SARS spike proteins. In the two left-hand blots (lanes 1-5), samples were boiled in SDS and β-mercaptoethanol; in the two right-hand blots (lanes 6-11), samples were in SDS only, with no boiling. Lanes 1-8 were labeled with a monoclonal raised against the His-tag protein; labes 9-11 were labeled with rabbit anti-SARS antibody.

FIGURE 46: Effect of SARS spike protein expression on cell viability.

FIGURE 47: Western blot analyses of COS7 cell lysates on 4% TG SDS gels showing oligomerization state of intracellular spike molecules. Blots were labeled with anti-His-tag mAb. The membrane fraction of COS7 cell lysate was fractionated by a sizing column before loading the lanes. Fractions 7 to 14 show bands with kDa values of: 71000, 1400, 898, 572, 365,232, 148 and 99, respectively.

FIGURE 48: Fractionation of cells into aqueous and detergent fractions.

FIGURE 49: Schematic of constructs for use in OMV preparation.

FIGURE 50: SARS HR1 and HR2 constructs.

FIGURE 51: Vaccine protection froms SARS in Balb/c mouse model.

FIGURE 52: Expressed on Spike protein in transfected 293 cell lysates (52A) or COS7 cell culture supernatants (52B). Proteins were separated on 4-20% TG SDS gels. The label was anti-His-tag, except for the right-hand three lanes of 52B, where the label was rabbit anti-SARS serum. In Figure 52A, the left-hand three lanes were treated with DTT and were boiled, but neither treatment was used for the right-hand three lanes. In Figure 52B, no DTT was used, but all lanes were heated to 80°C for 5 minutes.

FIGURE 53: Western blot of Spike proteins expressed in COS7 cells. Proteins were incubated at room temperature (RT), 80°C or 100°C to check for any effect on molecular weight. FIGURE 54 shows similar experiments on SARS virions.

FIGURE 55: Results of a pulse chase experiment, showing expression and processing of SARS spike protein following infection with alphavirus replicon particles. Cells were treated with or without EndoH as shown.

FIGURE 56: Effect of heating on Spike protein trimers.

FIGURE 57: Coomassie blue-stained gel of yeast-expressed proteins. Lanes are: 1-See Blue Standard (10μl); 2-pAB24 gbl (20μg); 3-SARS Spike S1 c.1 gbl (20μg); 4-SARS Spike S1 c.2 gbl (20μg); 5-See Blue Standard (10μl); 6-pAB24 ip (5μl); 7-SARS Spike S1 c.1 (5μl); 8-SARS Spike S1 c.2 (5μl).

FIGURES 58 to 64: Schematics of preparation of yeast expression constructs.

FIGURES 65 to 66: Yeast-expressed sequences for Spike.

FIGURE 67: Western blots showing expression of SARS spike protein from alphavirus replicon particles and replicon RNA. Figure 67A was run under non-reducing conditions and at room temperature (*i.e.* no heating), with lanes: (1) VEE/SIN-spike infection; (2) VEE/SIN-GFP infection; (3) Replicon-spike RNA transfection; (4) Replicon-GFP RNA transfection. Figure 67B was run with SARS virions at different temperatures, as shown.

FIGURE 68: induction of antibody responses in mice. Vaccine groups are: (1) Inactivated SARS Virus; (2) Truncated Recombinant Spike Protein; (3) Full length Spike: DNA+DNA.PLG+ Alphavirus; (4) Full length Spike: Alphavirus particles only.

FIGURE 69: Binding of human monoclonal antibody S3.2 to purified truncated Spike protein. The X-axis shows antibody concentration, and the Y-axis shows ELISA absorbance. The interpolation result is 2158.13.

FIGURE 70: Geometric mean ELISA titers of antibodies induced by the SARS-CoV spike protein delivered as different vaccines (left to right: inactivated virus; 3μg truncated spike protein; 75μg DNA encoding truncated spike protein.

FIGURE 71: Neutralization titers after immunization with (left) nSdΔTC protein or (right) DNA encoding nSdΔTC, delivered on PLG.

FIGURE 72: Correlation between the spike antigen binding and neutralizing antibodies

FIGURE 73: Western blot of CHO cell lines expressing Spike protein in full-length form (left) or in truncated form (right). Proteins were separated by 4-12% SDS-PAGE, with boiling in DTT and staining by polyclonal serum.

FIGURE 74: Structural components of SARS-CoV spike glycoprotein and expression construct. L denotes leader peptide (residues 1-13), TM the transmembrane, and Cy the cytoplasmic tail segments. The hexa-His tags are not shown.

FIGURE 75: Western blot analysis of SARS spike proteins expressed in COS7 cells. In Figure 75A, COS7 cells were transfected with indicated plasmid constructs and the expressed proteins in cell lysates 48 hr post-transfection were analysed by SDS-PAGE (4-20% polyacrylamide) in reducing and denaturing condtions, with proteins visualized by anti-histidine Mab. In Figure 75B, proteins were collected from cell culture medium 48 hr post-transfection and purified first by a ConA column and then by His-tag magnetic beads. Purifed proteins were analysed by SDS-PAGE (4-20% polyacrylamide) and were visualized by anti-SARS rabbit serum.

FIGURE 76: Endo H sensitivity of C-terminal truncated spike protein (SΔ) found in cell lysate (lanes 1,2) and culture medium (lanes 3,4). Positions of internal SΔ protein and secreted SΔ protein are marked with arrow heads.

FIGURE 77: Oligomeric status of the SARS spike protein. Recombinant S protein oligomer in COS7 cells transfected with the full-length spike construct (nSh). The cell lysates were treated with DTT and/or heat as indicated above each lane. The different forms of S protein in treated and untreated samples were visualized by SDS-PAGE (4% polyacrylamide) and Western blot analysis using anti-histidine MAb.

FIGURE 78: Effect of heat denaturation on the oligomeric status of recombinant S protein in the absence of DTT. The COS7 cell lysates were heated before the electrophoresis as indicated and the S proteins were visualized as described fogiFigure 77.

FIGURE 79: Effect of heat denaturation on the oligomeric status of spike protein in SARS virion particles. SARS-CoV were grown in Vero cells, purified and solubilized from the virion particles by SDS, heat-denatured as indicated and visualized as described in Figure 77, except that rabbit antiserum against the purified virus was used as a probe.

FIGURE 80: Analysis of the oligomeric status of SARS virion spike protein by cross-linking experiment. Solubilized SARS virion proteins were treated with DMS. Both untreated (–) and DMS treated (+) virion proteins were heat denatured in the absence of DTT and visualized by 4% PAGE followed by silver staining.

FIGURES 81 & 82: Analysis of the oligomeric status of truncated spike protein by heat denaturation. Truncated spike protein within COS7 cell lysates (81) or secreted into culture medium (82) were heat denatured as indicated in the absence of DTT and visualized by Western blot analysis.

FIGURE 83: Reactivity of deglycosylated full-length spike oligomer with conformational and non-conformational antibody. The full-length recombinant spike oligomer was partially deglycosylated with PNGase F in non denaturating condition and visualized by Western blot analysis using anti-histidine Mab (lane 1,2,3) or rabbit antiserum against purified SARS CoV (lane 4,5,6).

FIGURE 84: Localization of expressed SARS spike proteins in fractionated COS7 cell lysate visualized by western blot. Cells were transfected with indicated plasmids and lysed with Dounce homogeniser in hypotonic buffer 48 hr post transfection. Cell lysate was centrifuged to obtain soluble cytosol and insoluble membrane fraction that was further solublized by 4% Triton X-100. Proteins were heated with SDS at 80 C and analysed by SDS-PAGE (4-20% polyacrylamide) in reducing condtion. Proteins were visualized by anti-histidine Mab. The

cytosol fractions were loaded in lanes 1, 3, and 5 and the membrane fractions were loaded in lanes 2, 4, and 6.

FIGURE 85: Intracellular and surface expression of recombinant full-length (A,D) or truncated (B,E) spike protein in COS7 cells. The cells were fixed at 48 hrs posttransfection and either treated with detergent (Cytofix/perm, BD Biosciences) for intracellular immunofluorescence (A,B,C) or with 2% paraformaldehyde for cell surface immunofluorescence observation (D,E,F) at x40 magnification. Mock transfected cells (C,F) were included as controls.

FIGURES 86-105: SDS-PAGE od *E.coli* expressed proteins. Tot = total protein; Sol = soluble protein fraction. Labels are protein names (Tables 26-30).

FIGURE 106: Immunofluorescence after administration of vector encoding optimsed N antigen.

FIGURE 107: Immunofluorescence of (A) native and (B) codon-optimsed M sequences.

FIGURE 108: Immunofluorescence of (A) native and (B) codon optimsed E sequences.

FIGURES 109-111: Western blots of Vero cells using rabbit antibodies obtained after immunization with spike proteins expressed in *E.coli*.

FIGURE 112: Spike protein expression in 293 cells. Lanes: (M) Markers; (1) Mock transfected; (2,6) cells expressing nS protein, lysate; (3,7) cells expressing nSdTC protein, lysate; (4,8) cells expressing nS protein, supernatant; (5,9) (4) cells expressing nSdTC protein, supernatant. Staining antibody: (2 to 5) mouse serum obtained after DNA immunization; (6 to 9) rabbit serum obtained after immunization with whole killed virus.

FIGURE 113: Six reading frames of SEQ ID NO: 9968.

FIGURE 114: Six reading frames of SEQ ID NO: 10033.

FIGURE 115: Alignment of bovine coronavirus pol 1ab (top row; SEQ ID NO: 10068), avian infectious bronchitis pol 1ab (second row; SEQ ID NO: 10069), murine hepatitis virus pol 1ab (third row; SEQ ID NO: 10070), SEQ ID NO^S: 9997/9998 (fourth row) and a consensus sequence (bottom row; SEQ ID NO: 10071).

FIGURE 116: Schematic of coronavirus genome organization.

FIGURE 117: Schematic of coronavirus ORF1a/ORF1b gene products, including "*" region.

FIGURE 118: Alignment.

FIGURE 119: Alternative start codons within SEQ ID NO: 10080.

FIGURE 120: Six reading frames of SEQ ID NO: 10084.

FIGURE 121: Alignment of SEQ ID NO: 10033 and SEQ ID NO: 10084.

FIGURE 122: Reading frames in SEQ ID NO: 10084.

FIGURE 123: Start codon analysis for SEQ ID NO: 10084.

FIGURE 124: BLAST analysis of SEQ ID NO: 10210.

FIGURE 125: Epitope analysis of SEQ ID NO: 10210 by either (13A) Hopp & Woods or (13B) Kyte & Doolittle.

FIGURE 126: Reading frames in SEQ ID NO: 10299.

FIGURE 127: Reading frames in SEQ ID NO: 10505.

FIGURE 128: Reading frames in SEQ ID NO: 11563.

FIGURE 129: Reading frames in SEQ ID NO: 10033.

FIGURE 130: Alignment of SEQ ID NO: 9997 and SEQ ID NO: 10033.

FIGURE 131: Reading frames in SEQ ID NO: 10299.

FIGURE 132: Reading frames in SEQ ID NO: 10505.

FIGURE 133: Western Blot of SARS protease purification fractions.

FIGURE 134: Cleavage of DABCYL-EDANS (a fluorescent tagged peptide with a SARS protease cleavage site) by SARS protease at different concentrations. The graph shows activity/concentration correlations with no protease (♦), 0.95 uM protease (■) and 2.86 uM protease (●).

In the event of a discrepancy between a sequence in the sequence listing and a sequence in the drawings, the drawings should take precedence.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa., 19th Edition (1995); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel *et al.* eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream *et al.*, eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag); Peters and Dalrymple, *Fields Virology* (2d ed), Fields *et al.* (eds.), B.N. Raven Press, New York, NY.

All publications, patents and patent applications cited herein, are hereby incorporated by reference in their entireties.

Severe Acute Respiratory Syndrome (SARS) virus has recently been identified as a new viral species. The SARS viral species includes the following isolates.

- two virus isolates described in Peiris *et al.* "Coronavirus as a possible cause of severe acute respiratory syndrome" *Lancet* published online at *http://image.thelancet.com/extras/03art3477web.pdf* on April 8 2003, incorporated herein by reference in its entirety and the sequences deposited with GenBank at accession number AY268070.
- the isolates and viral sequences described in Drosten *et al.*, "Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome", New England Journal of Medicine, published online at *http://www.nejm.org* on April 10, 2003.
- the isolates and viral sequences described on the website of the WHO network on March 25 and 24, 2003.
- the isolates and viral sequences described in Tsang *et al.*, "A Cluster of Cases of Severe Acute Respiratory Syndrome in Hong Kong" *New England Journal of Medicine*, published online at *http://www.nejm.org* on March 31, 2003.
- the isolates and viral sequences described in Poutanen *et al.*, "Identification of Severe Acute Respiratory Syndrome in Canada" *New England Journal of Medicine*, published online at *http://www.nejm.org* on March 31, 2003.

As described in the *Lancet* article, a 646 base pair polynucleotide from the SARS virus has weak homology to viruses of the family *Cornoaviridae*. The *Lancet* article further reports that a deduced amino acid sequence (of 215 amino acids) from this sequence has about 57% sequence homology to the RNA polymerase of bovine coronavirus and murine hepatitis virus. Phylogenetic analysis of the protein sequences are also presented in the *Lancet* article showing that the polymerase sequence is most closely related to the group II coronaviruses.

Additional SARS viral isolates can be identified, isolated and/or sequenced by virologists skilled in the art. Virologists can readily identify new viral isolates as a SARS virus. Criteria which a virologist may use to identify new SARS isolates include: sequence homology of the new isolate to known SARS viral isolates; similar genomic organization of the new viral isolate to known SARS viral isolates; immunological (serologic) similarity or identity with known SARS viral isolates; pathology; and similarity of virion morphology with known SARS viral isolates; and similarity of infected cell morphology as that caused by known SARS viral isolates (visualized, for instance, by electron microscopy).

Methods for isolating and sequencing SARS viral isolates include the methods described by Peiris *et al.* in the *Lancet* paper. As reported in the *Lancet* paper, RNA from clinical samples

can be reverse transcribed with random hexamers and cDNA can be amplified with primers having sequences of SEQ ID NOS: 6584 & 6585 in the presence of 2.5 mmol/L magnesium chloride (94°C for 1 min, 50°C for 1 min, and 72°C for 1 min).

Reverse transcription of a viral isolate using random hexamers can be accomplished in an RT-PCR assay as follows. Virus isolates are propagated on mammalian cells, particularly fetal rhesus kidney cells. Total RNA from virus-infected and virus-uninfected fetal rhesus kidney cells is then isolated. RNA samples are reverse transcribed with a primer having SEQ ID NO: 6586. cDNA can be amplified by a primer having SEQ ID NO: 6587. Unique PCR products (in size) in the infected cell preparation are then cloned and sequenced, and genetic homology of the sequence compared with those in GenBank.

One skilled in the art would be able to identify and clone additional genomic regions using a variety of standard cloning techniques, such as, for example, using random primer RT-PCR and detection of sequences overlapping one or more of the above sequences, and/or using oligonucleotide primers, *e.g.*, degenerate primers, based on the sequences provided herein (see Figures 1-5, Figures 8-11, SEQ ID NOS: 3-20).

Cloning, sequencing and identification of SARS virus by one skilled in the art can be further facilitated by the use of polynucleotide sequences, particularly RNA polymerase sequences, from related Coronaviruses.

Sequence homology of new viral isolates with the known SARS isolates described above can be readily determined by one skilled in the art. New SARS isolates may be identified by a percent homology of viral nucleotide sequences of 99%, 95%, 92%, 90%, 85%, or 80% homology of the new virus to known SARS viral polynucleotide sequences. New SARS isolates may also be identified by percent homology of 99%, 95%, 92%, 90%, 85%, or 80% homology of the polypeptides encoded by the polynucleotides of the new virus and the polypeptides encoded by known SARS virus.

New SARS isolates may also be identified by a percent homology of 99%, 95%, 92%, 90%, 85%, or 80% homology of the polynucleotide sequence for specific genomic regions for the new virus with the polynucleotide sequence for specific genomic regions of the known SARS viruses. Additionally, new SARS isolates may be identified by a percent homology of 99%, 95%, 92%, 90%, 85%, or 80% homology of the polypeptide sequence encoded by the polynucleotide of specific genomic regions of the new SARS virus to the polypeptide sequence encoded by the polynucleotides of specific regions of the known SARS virus. These genomic regions may include regions (*e.g.*, gene products) which are typically in common among numerous coronaviruses, as well as group specific regions (*e.g.*, antigenic groups), such as, for example, any one of the following genomic regions which could be readily identified by a virologist skilled in the art: 5'untranslated region (UTR), leader sequence, ORF1a, ORF1b,

nonstructural protein 2 (NS2), hemagglutinin-esterase glycoprotein (HE) (also referred to as E3), spike glycoprotein (S) (also referred to as E2), ORF3a, ORF3b, ORF3x, nonstructural protein 4 (NS4), envelope (small membrane) protein (E) (also referred to as sM), membrane glycoprotein (M) (also referred to as E1), ORF5a, ORF5b, nucleocapsid phosphoprotein (N), ORF7a, ORF7b, intergenic sequences, 3'UTR, or RNA dependent RNA polymerase (pol). The SARS virus may have identifiable genomic regions with one or more the above-identified genomic regions. A SARS viral antigen includes a protein encoded by any one of these genomic regions. A SARS viral antigen may be a protein or a fragment thereof, which is highly conserved with coronaviruses. A SARS viral antigen may be a protein or fragment thereof, which is specific to the SARS virus (as compared to known cornaviruses). (See, Figures 1-5, Figures 8-11, SEQ ID NOS: 3-20).

One skilled in the art could also recognize electron microscopy of a SARS virus infected mammalian cell. Electron microscopy of SARS infected cells are shown in the *Lancet* paper. As discussed in the paper, electron microscopy of negative stained (3% potassium phospho-tungstate, pH 7.0) ultracentrifuged cell-culture extracts of SARS infected fetal rhesus kidney cells show the presence of pleomorphic enveloped virus particles of around 80-90 nm (range 70-130 nm) in diameter with surface morphology compatible with a coronavirus (see *Lancet* paper, Figure 1). Thin-section electron microscopy of infected cells reveals virus particles of 55-90 nm diameter within smooth walled vesicles in the cytoplasm (see *Lancet* paper, Figure 2B). Electron microscopy can also be used to observe virus particles at the cell surface. Electron microscopy of a human lung biopsy sample depicts similar viral morphology. See *Lancet* paper Figure 2A.

I. SARS POLYPEPTIDES AND POLYNUCLEOTIDES

The invention relates to nucleic acids and proteins from SARS virus. Such polynucleotides and polypeptides are exemplified further below.

In one embodiment, the polynucleotides of the invention do not include one of the following five primers, disclosed at *http://content.nejm.org/cgi/reprint/NEJMoa030781v2.pdf*: SEQ ID NOs: 6034-38.

The invention also includes polynucleotide sequences which can be used as probes for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in SEQ ID NOS: 21-1020. The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 21-1020.

The invention includes a polypeptide sequence comprising an amino acid sequence from the sequence shown in Figure 23. Such amino acid sequences are SEQ ID NOS: 6588-6809. The invention includes polypeptides comprising an amino acid sequence having sequence identity to these sequences, and the invention includes a fragment of a polypeptide comprising one of these sequences.

The invention includes a polypeptide comprising an amino acid sequence from the sequence shown in Figure 24. Such amino acid sequences are SEQ ID NOS: 6810-7179. The invention includes a protein comprising an amino acid sequence having sequence identity to these sequences, and the invention includes a fragment of a protein comprising one of these sequences.

The invention includes a protein comprising SEQ ID NO: 6039. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 6039. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 6039. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6039, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 6039, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 6039, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6039, or a fragment thereof. SEQ ID NO: 6039 demonstrates functional homology with ORF1a of coronaviruses.

Predicted transmembrane or hydrophobic regions of SEQ ID NO: 6039 are identified below. Although the polyprotein of coronaviruses is proteolytically cleaved into numerous smaller proteins, hydrophobic domains in the polyprotein are known to mediate the membrane association of the replication complex and to be able to dramatically alter the architecture of host cell membranes. Accordingly, the hydrophobic domains of the polyprotein are targets for genetic mutation to develop attenuated SARS virus vaccines. The hydrophobic domains are also targets for small molecule inhibitors of the SARS virus. The hydrophobic domains may also be used to generate antibodies specific to those regions to treat or prevent SARS virus infection.

Predicted Transmembrane Helices in SEQ ID NO: 6039

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

Inside to outside helices : 43 found

from		to		score	center
100	(100)	118	(116)	103	107
473	(473)	488	(488)	1003	481
529	(532)	549	(549)	541	539
584	(584)	606	(601)	1049	594
773	(773)	791	(789)	514	782

Outside to inside helices : 43 found

from		to		score	center
94	(97)	118	(112)	291	104
400	(400)	418	(415)	243	407
473	(473)	488	(488)	1113	481
523	(528)	548	(548)	285	538
583	(583)	606	(601)	662	593

1071	(1071)	1089	(1086)	243	1078
1121	(1121)	1137	(1137)	459	1130
1679	(1679)	1696	(1696)	404	1686
2098	(2102)	2119	(2116)	509	2109
2145	(2145)	2160	(2160)	797	2153
2206	(2209)	2224	(2224)	2686	2216
2316	(2316)	2332	(2332)	2123	2325
2335	(2339)	2358	(2354)	2101	2346
2373	(2373)	2390	(2390)	532	2380
2597	(2600)	2615	(2615)	307	2607
2753	(2753)	2770	(2768)	2242	2760
2831	(2833)	2854	(2851)	759	2841
2879	(2882)	2900	(2897)	526	2889
2990	(2996)	3012	(3010)	1289	3003
3024	(3024)	3042	(3039)	2281	3032
3054	(3058)	3075	(3072)	2536	3065
3105	(3109)	3127	(3123)	2010	3116
3143	(3143)	3163	(3159)	184	3152
3253	(3255)	3272	(3272)	319	3262
3346	(3346)	3366	(3366)	203	3356
3375	(3375)	3392	(3392)	305	3384
3438	(3438)	3455	(3453)	1021	3445
3559	(3567)	3584	(3581)	1885	3574
3589	(3589)	3606	(3604)	2018	3596
3611	(3611)	3629	(3629)	2304	3621
3659	(3659)	3674	(3674)	1561	3667
3756	(3758)	3777	(3774)	2352	3767
3890	(3890)	3904	(3904)	485	3897
3916	(3919)	3934	(3934)	241	3926
4035	(4035)	4051	(4051)	335	4044
4217	(4217)	4232	(4232)	272	4224
4239	(4239)	4257	(4254)	402	4247

776	(776)	791	(791)	1435	783
1068	(1071)	1089	(1086)	370	1078
1121	(1121)	1137	(1137)	455	1130
1679	(1679)	1696	(1694)	340	1686
2098	(2098)	2119	(2116)	678	2109
2148	(2148)	2163	(2163)	434	2155
2208	(2210)	2231	(2226)	2389	2219
2309	(2309)	2332	(2326)	1773	2318
2342	(2342)	2368	(2360)	1666	2353
2373	(2373)	2390	(2390)	254	2380
2753	(2755)	2770	(2770)	2119	2763
2832	(2835)	2854	(2851)	687	2844
2858	(2858)	2873	(2873)	253	2866
2879	(2882)	2899	(2899)	400	2889
2990	(2990)	3005	(3005)	875	2998
3020	(3024)	3042	(3042)	2795	3032
3059	(3059)	3075	(3075)	2137	3067
3105	(3108)	3127	(3123)	1902	3115
3142	(3145)	3162	(3162)	540	3152
3343	(3351)	3366	(3366)	496	3358
3437	(3437)	3453	(3453)	848	3444
3489	(3491)	3508	(3505)	302	3498
3560	(3560)	3577	(3577)	1460	3569
3591	(3591)	3606	(3606)	2193	3598
3610	(3610)	3627	(3627)	1484	3620
3656	(3658)	3678	(3675)	1240	3668
3681	(3684)	3701	(3699)	590	3691
3710	(3713)	3738	(3728)	1696	3721
3723	(3723)	3738	(3738)	1670	3730
3760	(3760)	3777	(3775)	2367	3767
3881	(3884)	3902	(3900)	249	3892
4099	(4099)	4114	(4114)	389	4106
4234	(4234)	4254	(4249)	325	4241
4338	(4341)	4360	(4360)	505	4348

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6039, wherein said fragment comprises an amino acid sequence including one or more of the hydrophobic transmembrane sequences identified above. The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6039 wherein said fragment comprises one or more of the following polypeptide sequences of SEQ ID NO: 6039: 473-488, 529-549, 584-606, 773-791, 2098-2119, 2145-2160, 2206-2224, 2316-2332, 2335-2358, 2373-2390, 2753-2770, 2831-2854, 2879-2900, 2990-3012, 3024-3042, 3054-3075, 3105-3127, 3438-3455, 3559-3584, 3589-3606, 3611-3629, 3659-3674, 3756-3777, 473-488, 583-606, 776-791, 2098-2119, 2208-2231, 2309-2332, 2342-2368, 2753-2770, 2832-2854, 2990-3005, 3020-3042, 3059-3075, 3105-3127, 3142-3162, 3437-3453, 3560-3577, 3591-3606, 3610-3627, 3656-3678, 3710-3738, 3723-3738, and 3760-3777. Preferably, the fragment comprises one or more of the following polypeptide sequences of SEQ ID NO: 6039: 2206-2224, 2316-2332, 2335-2358, 2753-2770, 3024-3042, 3054-3075, 3105-3127, 3589-3606, 3611-3629, 3756-3777, 2208-2231, 2753-2770, 3020-3042, 3059-3075, and 3591-3606. Preferably, the fragment comprises one or more of the following

polypeptide sequences of SEQ ID NO: 6039: 2206-2224 and 3020-3042. The invention also includes polynucleotides encoding each of the polypeptide fragments identified above.

The invention includes an attenuated SARS virus wherein said attenuated SARS virus contains an addition, deletion or substitution in the polynucleotides encoding for one of the hydrophobic domains identified above. The invention also includes a method for creating an attenuated SARS virus comprising mutating a SARS virus by adding, deleting or substituting the viral genome of the SARS virus to alter the coding of one or more of the hydrophobic domains of SEQ ID NO: 6039 identified above.

The invention includes an antibody which specifically identifies one or more of the hydrophobic regions of SEQ ID NO: 6039 identified above. The invention includes a small molecule which binds to, interferes with the hydrophobicity of or otherwise disrupts one or more of the hydrophobic regions of SEQ ID NO: 6039 identified above.

Predicted N-glycosylation sites of SEQ ID NO: 6039 are identified in the chart below.

Prediction of N-glycosylation sites in SEQ ID NO: 6039

Position			Potential	Jury agreement	NGlyc result	
48	NGTC	SEQ ID NO: 7180	0.6371	(7/9)	+	
389	NHSN	SEQ ID NO: 7181	0.6132	(6/9)	+	
916	NFSS	SEQ ID NO: 7182	0.5807	(7/9)	+	
1628	NHTK	SEQ ID NO: 7183	0.5610	(7/9)	+	
1696	NKTV	SEQ ID NO: 7184	0.5297	(5/9)	+	
2031	NPTI	SEQ ID NO: 9764	0.5299	(5/9)	+	WARNING: PRO-X1.
2249	NSSN	SEQ ID NO: 7185	0.6329	(9/9)	++	
2459	NPTD	SEQ ID NO: 9765	0.5599	(6/9)	+	WARNING: PRO-X1.
2685	NVSL	SEQ ID NO: 7186	0.6071	(8/9)	+	
4233	NATE	SEQ ID NO: 7187	0.6144	(7/9)	+	

Accordingly, the invention comprises a fragment of SEQ ID NO: 6039 wherein said fragment comprises an amino acid sequence which includes one or more of the N-glycosylation sites identified above. Preferably, the fragment comprises one or more sequences selected from the group consisting of SEQ ID NOS: 7180-7187 & 9764-9765. Preferably, the fragment comprises the amino acid sequence NSSN (SEQ ID NO: 7185).

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6039 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6039 are identified in Table 13. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified as SEQ ID NOS: 7400-7639; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the

polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified as SEQ ID NOS: 7400-7639, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus.

The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The ORF1a and ORF1b sequences of coronaviruses are typically translated as a single ORF1ab polyprotein. Slippage of the ribosome during translation generates an a-1 frameshift. One region of such slippage is illustrated below:

```
    gggttttacacttagaaacacagtctgtaccgtctgcggaatgtggaaaggttatggctgtagttgtga
+1    G  F  T  L  R  N  T  V  C  T  V  C  G  M  W  K  G  Y  G  C  S  C  D
+3   G  F  Y  T  -  K  H  S  L  Y  R  L  R  N  V  E  R  L  W  L  -  L  -
    ccaactccgcgaacccttgatgcagtctgcggatgcatcaacgttttaaacgggtttgcggtgtaagt
+1    Q  L  R  E  P  L  M  Q  S  A  D  A  S  T  F  L  N  G  F  A  V  -  V
+3   P  T  P  R  T  L  D  A  V  C  G  C  I  N  V  F  K  R  V  C  G  V  S
    gcagcccgtcttacaccgtgcggcacaggcactagtactg  (SEQ ID NO: 7224)
+1    Q  P  V  L  H  R  A  A  Q  A  L  V  L   (SEQ ID NOS: 7225-7226)
+3   A  A  R  L  T  P  C  G  T  G  T  S  T    (SEQ ID NOS: 7227-7229)
```

which would generate the following translational slippage (SEQ ID NOS: 7230-7231):

```
ccaactccgcgaacccttgatgcagtctgcggatgcatcaacgttttaaacgggtttgcggtgtaagt
 Q  L  R  E  P  L  M  Q  S  A  D  A  S  T  F  L  N R  V  C  G  V  S
```

Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 7232. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 7232. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 7232 The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 7232 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 7232 or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 7232 or a

fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 7232 or a fragment thereof.

The invention also includes a polypeptide comprising amino acid sequence X_1-X_2-X_3, where X_1 is SEQ ID NO: 7233, X_2 is from one to ten amino acids, and X_3 is SEQ ID NO: 7234. X_2 can comprise any sequence of one to ten amino acids (SEQ ID NOS: 7235-7244) but, in preferred embodiments, X_2 is selected from the group consisting of F, FL, FLN, FLNR (SEQ ID NO: 7245), FLNRV (SEQ ID NO: 7246) and FLNRVC (SEQ ID NO: 7247). Preferably, X_2 is SEQ ID NO: 7247. These preferred embodiments are shown as SEQ ID NOS: 7248-7253.

The invention includes a polypeptide comprising an amino acid sequence having sequence identity to said amino acid sequences X_1-X_2-X_3. The invention includes a fragment of a polypeptide comprising said amino acid sequences X_1-X_2-X_3. The invention includes a diagnostic kit comprising a polypeptide comprising said amino acid sequences X_1-X_2-X_3 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding said amino acid sequences X_1-X_2-X_3 or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising said amino acid sequences X_1-X_2-X_3 or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising said amino acid sequences X_1-X_2-X_3 or a fragment thereof.

The amino acid sequences X_1-X_2-X_3 (*i.e.* SEQ ID NOS: 7235-7244) demonstrate functional homology with the polyprotein of murine hepatitis virus. This polyprotein is cleaved to produce multiple proteins. Proteins which can be generated from the X_1-X_2-X_3 polyprotein, where X_2 is six amino acids (SEQ ID NO: 7240) are listed below.

Mouse virus protein	Coordinates in Mouse virus	Coordinates in SEQ ID NO: 7240
Nsp2	3334-3636	3241-3546
Nsp3	3637-3923	3547-3836
Nsp4	3924-4015 (or 4012)	3837-3919
Nsp5	4016 (or 4013)-4209	3920-4117
Nsp6	4210-4319	4118-4230
Nsp7	4320-4456	4231-4369
Nsp9	4457-5384	4370-5301
Nsp10	5385-5984	5302-5902
Nsp11	5985-6505	5903-6429
Nsp12	6506-6879	6430-6775
Nsp13	6880-7178	6776-7073

The invention includes a fragment of the amino acid sequence X_1-X_2-X_3 (*i.e.* SEQ ID NOS: 7235-7244) wherein the fragment comprises one of the polypeptide sequences identified in the above table. The invention further includes a fragment of the amino acid sequence X_1-X_2-X_3 wherein said fragment comprises a polypeptide sequence which has a serine at its N-terminus and a glutamine at its C-terminus. The invention further includes a fragment of the amino acid sequence X_1-X_2-X_3 wherein said fragment comprises a polypeptide sequence which has an

Alanine at its N-terminus and a glutamine at its C-terminus. The invention further includes a fragment of the amino acid sequence X_1-X_2-X_3 wherein said fragment comprises a polypeptide sequence which has a Asparagine at its N-terminus and a glutamine at its C-terminus. The invention further includes a fragment of the amino acid sequence X_1-X_2-X_3 wherein said fragment comprises a Cysteine at its N-terminus and a Glutamine at its C-terminus. Each of the fragments identified above can be used in fusion proteins.

The invention includes a diagnostic kit comprising a polypeptide comprising at least one of the fragments of the amino acid sequence X_1-X_2-X_3 (*i.e.* SEQ ID NOS: 7235-7244) identified in the above paragraph. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding at least one of the fragments of the amino acid sequence X_1-X_2-X_3 identified in the above paragraph. The invention includes an immunogenic composition comprising a polypeptide comprising at least one of the fragments of the amino acid sequence X_1-X_2-X_3 identified in the above paragraph. The invention includes an antibody which recognizes a polypeptide comprising at least one of the fragments of the amino acid sequence X_1-X_2-X_3 identified in the above paragraph.

Predicted N-glycosylation sites of the amino acid sequence X_1-X_2-X_3 when X_2 is six amino acids are identified at the asparagines located at the following amino acid positions 48; 389; 556; 916; 1628; 1696; 1899; 2079; 2249; 2252; 2507; 2685; 3303; 3373; 3382; 3720; 4150; 4233; 4240; 5016; 5280; 5403; 5558; 5650; 5905; 6031; 6130; 6474; 6918; 6973. Accordingly, the invention comprises a fragment of SEQ ID NO: 7239 wherein said fragment is at least ten amino acids and wherein said fragment comprises one or more of the asparagines from the amino acid positions of SEQ ID NO: 7239 selected from the group consisting of 8; 389; 556; 916; 1628; 1696; 1899; 2079; 2249; 2252; 2507; 2685; 3303; 3373; 3382; 3720; 4150; 4233; 4240; 5016; 5280; 5403; 5558; 5650; 5905; 6031; 6130; 6474; 6918; and 6973.

A zinc binding region 2 site within SEQ ID NOS: 7235-7244 is identified at amino acid residues 2102-2112 (SEQ ID NO: 7254 HGIAAINSVPW). The polypeptide of SEQ ID NOS: 7235-7244 will be processed by the SARS virus into multiple peptides. This zinc binding region falls within the nsp1 region of the polypeptide. SEQ ID NO: 7254 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 7254. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 7254. The invention includes a method of screening SEQ ID NO: 7254 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 7254 in a host cell. The invention includes a fragment of SEQ ID NOS: 7235-7244, wherein said fragment comprises SEQ ID NO: 7254. The invention includes a polypeptide comprising SEQ ID NO: 7254 wherein said polypeptide is complexed with a zinc ion. The invention includes a small molecule which

prevents a zinc ion from complexing with the polypeptide of SEQ ID NO: 7254. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 7254.

The polyprotein encoded by the SARS virus will contain at least two protease domains: a papain-like cystein protease (PLP) and a chymotrypsin-picornavirus 3C-like protease (3CLp). (There may be more than one copy of the PLP domain). These proteases function to cleave the polyprotein into multiple smaller proteins. The 3C-like protease, also known as the "main protease" or Mpro, is itself cleaved from the polyprotein by its own autoprotease activity. See generally, Chapter 35 of *Fields Virology* (2nd ed), Fields *et al.* (eds.), B.N. Raven Press, New York, NY, and Anand *et al.*, *EMBO Journal* (2002) 21 (13): 3213-3224. This 3CLp generally corresponds with the Nsp2 region identified above.

The SARS virus 3CLp protein is further characterized by SEQ ID NO: 6569 (also SEQ ID NO: 9769), as shown in FIGURE 15.

FIGURE 16 also illustrates the SARS virus 3CLp, in allignment with the 3CLp of avian infectious bronchitis (IBV; SEQ ID NO: 6570), mouse hepatitis virus (MHV; SEQ ID NO: 6571), and bovine coronavirus (BCoV; SEQ ID NO: 6572). Accordingly, the invention includes a polypeptide sequence comprising SEQ ID NO: 6569, or a fragment thereof, or a polypeptide sequence having sequence identity thereto. The invention further includes a polynucleotide sequence encoding SEQ ID NO: 6569, or a fragment thereof. The invention includes a polynucleotide sequence encoding a polypeptide sequence having sequence identity to SEQ ID NO: 6569.

The invention further includes a method of screening for an inhibitor of the SARS virus 3CLp protein. In one embodiment, the invention includes a method of screening for an inhibitor of SEQ ID NO: 6569. The invention includes a method of recombinantly expressing the SARS virus 3CLp protein in a host cell. The invention includes a method of recombinantly expressing a polypeptide sequence comprising SEQ ID NO: 6569 or an enzymatically active fragment thereof or a polypeptide sequence having sequence identity thereto. The invention includes a small molecule which inhibits or reduces the proteolytic activity of the SARS virus 3CLp protein. The invention includes a small molecule which inhibits or reduced the proteolytic activity of the polypeptide comprising SEQ ID NO: 6569.

Catalytic residues of the SARS virus 3CLp are identified in FIGURE 15 and 16. Specifically, a catalytic histidine and a catalytic cysteine are identified. Such catalytic sites are targets for small molecules which could inhibit or reduce the protease activity of 3CLp. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6569, wherein said fragment comprises at least one catalytic site. Preferably, the catalytic site is selected from the group consisting of the indicated catalytic histidine and the catalytic cysteine in FIGURE 15 and 16. The invention includes a polynucleotide encoding a polypeptide, wherein

said polypeptide comprises a fragment of SEQ ID NO: 6569, wherein said fragment comprises at least one catalytic site. Preferably, the catalytic site is selected from the group consisting of the indicated catalytic histidine and the catalytic cysteine.

The invention further includes a method of screening a compound library to identify a small molecule which inhbits a catalytic site of a SARS virus 3CLp. Preferably, the 3CLp comprises SEQ ID NO: 6569, or a fragment thereof, or a sequence having sequence identity thereto. The catalytic site is preferably selected from the group consisting of the indicated catalytic histidine and the catalytic cysteine in FIGURE 15 and 16.

The invention includes a small molecule which inhibits the catalytic site of a SARS virus 3CLp. Preferably, the 3CLp comprises SEQ ID NO: 6569, or a fragment thereof, or a sequence having sequence identity thereto. The catalytic site is preferably selected from the group consisting of the indicated catalytic histidine and the catalytic cysteine in FIGURE 15 and 16.

Residues of the substrate site of the SARS virus 3CLp are identified in FIGURE 15 and 16. Specifically, a substrate site is indicated at a phenylalanine, a tyrosine and a histidine. Such substrate sites are targets for small molecules which could inhibit or reduce the protease activity of 3CLp. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6569, wherein said fragment comprises at least one substrate site. Preferably, the substrate site is selected from the group consisting of the indicated substrate phenylalanine, tyrosine and histidine in FIGURE 15 and 16. The invention includes a polynucleotide encoding a polypeptide, wherein said polypeptide comprises a fragment of SEQ ID NO: 6569, wherein said fragment comprises at least one substrate site. Preferably, the substrate site is selected from the group consisting of the indicated substrate phenylalanine, tyrosine and histidine in FIGURE 15 and 16.

The invention further includes a method of screening a compound library to identify a small molecule which blocks a substrate site of a SARS virus 3CLp. Preferably, the 3CLp comprises SEQ ID NO: 6569, or a fragment thereof, or a sequence having sequence identity thereto. The substrate site is preferably selected from the group consisting of the indicated substrate phenylalanine, tyrosine and histidine in FIGURE 15 and 16.

The invention includes a small molecule which inhibits the substrate site of a SARS virus 3CLp. Preferably, the 3CLp comprises SEQ ID NO: 6569, or a fragment thereof, or a sequence having sequence identity thereto. The substrate site is preferably selected from the group consisting of the indicated substrate phenylalanine, tyrosine and histidine in FIGURE 15 and 16.

The invention further includes a diagnostic kit comprising a polynucleotide encoding a SARS virus 3CLp or a fragment thereof. Preferably, the SARS virus 3CLp comprising SEQ ID NO: 6569 or a fragment thereof or a polypeptide sequence having sequence identity thereto. Preferably, the fragment comprising one or more sites selected from the group consisting of a

catalytic site and a substrate site. Preferably, the catalytic site is selected from the group consisting of one or more of the sites identified in FIGURE 15 and 16. Preferably, the substrate site is selected from the group consisting of one or more of the sites identified in FIGURE 15 and 16.

The invention further comprises a diagnostic kit comprising an antibody specific to a SARS virus 3CLp or a fragment thereof. Preferably, the antibody is specific to the polypeptide comprising SEQ ID NO: 6569 or a fragment thereof or a polypeptide sequence having sequence identity thereto. Preferably, the antibody is specific to one or more sites of a SARS virus 3CLp selected from the group consisting of a catalytic site and a substrate site. Preferably, the catalytic site is selected from the group consisting of one or more of the sites identified in FIGURE 15 and 16. Preferably, the substrate site is selected from the group consisting of one or more of the sites identified in FIGURE 15 and 16.

The invention includes a polypeptide comprising an amino acid sequence from the sequence shown in Figure 25. The two amino acid sequences within Figure 25, separated by a *, are SEQ ID NOS: 7188 & 7189. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to the figure 25 translation. The invention includes a fragment of a polypeptide comprising the figure 25 sequence. The invention includes a diagnostic kit comprising a polypeptide comprising the figure 25 translation, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding the figure 25 translation, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising the figure 25 translation, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising the figure 25 sequence, or a fragment thereof. The figure 25 sequence demonstrates functional homology with ORF1b of coronaviruses.

SEQ ID NO: 7188 is an open reading frame within Figure 25. The invention includes a polypeptide comprising SEQ ID NO: 7188. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 7188. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 7188. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 7188, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 7188, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 7188, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 7188, or a fragment thereof.

SEQ ID NO: 7190 is an open reading frame within SEQ ID NO: 7188. The invention includes a polypeptide comprising SEQ ID NO: 7190, a fragment thereof or a polypeptide having sequence identity thereto. The invention further includes a polynucleotide encoding SEQ

ID NO: 7190, a fragment thereof or a polypeptide sequence having sequence identity thereto. An example of a polynucleotide encoding SEQ ID NO: 7190 is given as SEQ ID NO: 7191.

SEQ ID NO: 7188 also contains an open reading frame comprising SEQ ID NO: 6042. The invention includes a polypeptide comprising SEQ ID NO: 6042. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 6042. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 6042. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6042, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 6042, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 6042, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6042, or a fragment thereof. SEQ ID NO: 6042 demonstrates functional homology to a coronavirus spike protein.

Predicted transmembrane regions of SEQ ID NO: 6042 are identified below.

Predicted Transmembrane helices of SEQ ID NO: 6042

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

Inside to outside helices : 18 found

from		to		score	center
1	(1)	16	(16)	959	9
233	(237)	257	(252)	905	244
345	(347)	364	(361)	490	354
345	(354)	369	(369)	420	362
497	(497)	513	(513)	239	506
573	(573)	588	(588)	811	580
645	(648)	666	(663)	302	656
690	(696)	714	(711)	428	704
857	(860)	882	(874)	1508	867
1031	(1031)	1046	(1046)	446	1039
1199	(1203)	1219	(1217)	2667	1210

Outside to inside helices : 13 found

from		to		score	center
1	(1)	17	(17)	684	10
222	(222)	240	(237)	238	229
244	(247)	264	(264)	613	254
349	(355)	369	(369)	314	362
496	(496)	511	(511)	488	503
573	(573)	591	(591)	712	581
650	(652)	666	(666)	474	659
674	(679)	702	(696)	190	686
691	(696)	713	(711)	210	704
866	(868)	886	(886)	1172	876
1198	(1201)	1215	(1215)	3221	1208

SEQ ID NO: 6042, the spike protein, is a surface exposed polypeptide. Recombinant expression of a protein can be hindered by hydrophobic transmembrane regions. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 6042 wherein one or more of the hydrophobic regions identified above is removed. The invention further includes a polynucleotide encoding such a polypeptide. The invention includes recombinantly expressing the protein in a host cell. Primers for amplifying the gene for spike protein and fragments thereof, such as fragments encoding the soluble ectodomain, include SEQ ID NOS: 9753-9763 (Xiao *et al.* (2003) *Biochem Biophys Res Comm* 312:1159-1164).

Further characterization of SEQ ID NO: 6042 is set forth below.

PSORT --- Prediction of Protein Localization Sites

```
version 6.4(WWW)
SEQ ID NO: 6042 - 1255 Residues
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 2
     Position of the most N-terminal TMS: 496 at i=2
MTOP: membrane topology (Hartmann et al.)
     I(middle): 503   Charge diffirence(C-N):  1.0
McG: Examining signal sequence (McGeoch)
     Length of UR:   13
     Peak Value of UR:   3.28
     Net Charge of CR: 0
     Discriminant Score:      8.66
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 5.94
     Possible cleavage site: 13
>>> Seems to have a cleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 14
ALOM new cnt: 1 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 1  value: -12.26  threshold: -2.0
     INTEGRAL    Likelihood =-12.26   Transmembrane 1202-1218 (1194-1228)
     PERIPHERAL  Likelihood =  0.16
     modified ALOM score:   2.55
>>> Seems to be a Type Ia membrane protein
     The cytoplasmic tail is from 1219 to 1255 (37 Residues)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
(14) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 14
     Uncleavable? Ipos set to: 24
Discrimination of mitochondrial target seq.:
     positive ( 2.18)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 24)  from: 1  to: 10  Score:  8.0
SKL motif (signal for peroxisomal protein):
     pos: 964(1255), count: 1   SRL
     SKL score (peroxisome):  0.1
Amino Acid Composition Tendency for Peroxisome:   1.37
     AAC not from the N-term., score modified
Peroxisomal proteins?  Status: notclr
     AAC score (peroxisome): 0.079
Amino Acid Composition tendency for lysosomal proteins
     score:  0.39  Status: notclr
GY motif in the tail of typeIa? (lysosomal)
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
```

```
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Type Ia is favored for plasma memb. proteins
Checking the NPXY motif..
Checking the YXRF motif..
Checking N-myristoylation..

----- Final Results -----
plasma membrane --- Certainty= 0.460(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.171(Affirmative) < succ>
endoplasmic reticulum (membrane) --- Certainty= 0.100(Affirmative) < succ>
endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>
```

SEQ ID NO: 6042 appears to have a N-terminus signaling region, followed by a surface exposed region, followed by a transmembrane region followed by a C-terminus cytoplasmic domain region. Accordingly, the invention includes an immunogenic, surface exposed fragment of SEQ ID NO: 6042. Preferably, said fragment comprises an amino acid sequence which does not include the last 50 amino acids of the C-terminus of SEQ ID NO: 6042. Preferably, said fragment comprises an amino acid sequence which does not include the last 70 amino acids of the C-terminus of SEQ ID NO: 6042. Preferably, said fragment does not include a transdomain region of SEQ ID NO: 6042. Preferably, said fragment does not include a C-terminus cytoplasmic domain of SEQ ID NO: 6042. Preferably, said fragment does not include a N-terminus signal sequence. Preferably, said fragment does not include amino acids 1-10 of the N-terminus of SEQ ID NO: 6042. Preferably, said fragment does not include amino acids 1-14 of the N-terminus of SEQ ID NO: 6042. Two oligopeptide fragments of SEQ ID NO: 6042 that are able to elicit anti-spike antibodies are SEQ ID NOS: 7398 & 7399, as described (with additional C-terminus cysteines) by Xiao *et al.* (2003) *Biochem Biophys Res Comm* 312:1159-1164. C-terminal truncations of spike protein, with removal of part of the cytoplasmic region, or removal upto and including the transmembrane region, are described by Yang *et al.* (2004) *Nature* 428:561-564.

A variant of SEQ ID NO: 6042 that is included within the invention is SEQ ID NO: 9962. Compared to SEQ ID NO: 6042, this sequence has Ser at residue 581 instead of Ala, and has Phe at residue 1152 instead of Leu.

The spike protein of coronaviruses may be cleaved into two separate chains into S1 and S2. The chains may remain associated together to form a dimer or a trimer. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 6042 wherein said polypeptide has been cleaved into S1 and S2 domains. The invention further includes a polypeptide comprising SEQ ID NO: 6042 wherein amino acids 1-10, preferably amino acids 1-14 of the N-terminus are removed and further wherein SEQ ID NO: 6042 is cleaved into S1 and S2 domains. Preferably the polypeptide is in the form of a trimer.

The spike protein appears to form an alpha-helical structure in the transmembrane region of the protein, preferably in the S2 domain. This alpha-helical structure is thought to associate with at least two additional spike proteins to form a trimer. Helical or coiled regions of the spike protein are identified below. Predicted coiled-coils of SEQ ID NO: 6042 (spike protein) are at amino acids 900-1005 and 1151-1185 (see Figure 12).

Accordingly, the invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 6042 wherein said fragment includes a coiled region of SEQ ID NO: 6042. Said fragment preferably includes the amino acid sequences selected from the group consisting of amino acid positions 900 to 1005 and amino acid positions 1151 to 1185 of SEQ ID NO: 6042. The invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 6042, wherein said fragment does not include a coiled region of SEQ ID NO: 6042. Said fragment preferably includes the amino acid sequences selected from the group consisting of amino acid positions 900 to 1005 and amino acid positions 1151 and 1185 of SEQ ID NO: 6042.

The spike protein is believed to play an integral role in fusion and infection of Coronaviruses with mammalian host cells. Analysis of coronavirus spike proteins as well as similar surface proteins in other viruses has identified at least two structural motifs, typically located within the S2 domain, associated with this fusion event: heptad repeats (HR) and membrane fusion peptides.

At least two 4,3 hydrophobic heptad repeat (HR) domains are typically found in the ectodomain of the S2 domain of Coronaviruses. One heptad repeat region (HR1) is typically located adjacent to a fusion peptide while a second heptad region (HR2) is typically located near the C-terminus of the S2 domain, close to the transmembrane anchor. Heptad repeats are characteristic of coiled-coil structures and the heptad repeats found in viral surface proteins (such as coronavirus spike protein) are thought to form bundled helix structures which are involved in viral entry. *See* Bosch *et al.*, *J. Virology* (2003) 77:8801-8811 (Figure 1B of this reference illustrates an alignment of the HR1 and HR2 regions of five coronaviruses along with SARS, annotated "HCov-SARS").

Heptad repeats generally contain a repeating structure of seven amino acids, designated *a-b-c-d-e-f-g*, where hydrophobic sidechains of residues *a* and *d* typically form an apolar stripe, and electrostatic interactions are found in residues *e* and *g*. Position *a* is most frequently Leu, Ile or Ala and position *d* is usually Leu or Ala. Residues *e* and *g* are often Glu or Gln, with Arg and Lys also prominent at position *g*. Charged residues are common to positions *b*, *c* and *f* as these residues may be in contact with solvent. Exceptions to these general parameters are known. For instance Pro residues are sometimes found within the heptad.

The HR1 and HR2 sequences of an MHV strain have been postulated to assemble into a thermostable, oligomeric, alphahelical rold-like complex, with the HR1 and HR2 helices

oriented in an antiparallel manner. *Id.* In this same study, HR2 was asserted to be a strong inhitibor of both virus entry into the cell and cell-cell fusion.

HR1 and HR2 sequences have been identified in the SARS virus genome. The SARS virus HR1 region comprises approximately amino acids 879 to 1005 of SEQ ID NO: 6042 or fragments thereof capable of forming at least one alpha-helical turn. Preferably, said fragments comprise at least 7 (*e.g.*, at least 14, 21, 28, 35, 42, 49 or 56) amino acid residues. SEQ ID NO: 7192, includes amino acids 879 to 1005 of SEQ ID NO: 6042.

A preferred fragment of HR1 comprises amino acid residues 879 to 980 of SEQ ID NO: 6042. This preferred fragment is SEQ ID NO: 7193.

Another preferred fragment of HR1 comprises amino acid residues 901 to 1005 of SEQ ID NO: 6042. This preferred fragment is SEQ ID NO: 7194.

The SARS virus HR2 region comprises approximately amino acids 1144 to 1201 of SEQ ID NO: 6042, or fragments thereof capable of forming at least one alpha-helical turn. Preferably, said fragments comprise at least 7 (*e.g.*, at least 14, 21, 28, 35, 42, 49 or 56) amino acid residues. SEQ ID NO: 7195 includes amino acids 1144 to 1201. A preferred fragment of HR2 comprises amino acids 1144 to 1195 of SEQ ID NO: 6042. This preferred fragment is SEQ ID NO: 7196.

Membrane Fusion peptides sequences within the spike protein are also believed to participate in fusion (and infection) of the virus with a host cell. Fusion peptides generally comprise about 16 to 26 amino acid residues which are conserved within viral families. These Membrane Fusion peptides are relatively hydrophobic and generally show an asymmetric distribution of hydrophobitiy when modeled into an alpha helix. They are also generally rich in alanine and glycine.

At least three hydrophobic Membrane Fusion peptide regions have been identified within coronaviruses (PEP1, PEP2, and PEP3). *See,* Luo *et al.*, "Roles in Cell-Cell Fusion of Two Conserved Hydrophobic Regions in the Murine Coronavirus Spike Protein", Virology (1998) 244:483-494. Figure 1 of this paper shows an alignment of Membrane Fusion peptide sequences of Mouse Hepatitis Viris, Bovine Corona Virus, Feline Infectious Peritonitis Virus, Transmissible Gastroenteritis Virus and Infectious Bronchitis Virus. *See also*, Bosch *et al.*, "The Coronavirus Spike Protein is a Class I Virus Fusion Protein: Structural and Functional Characterization of the Fusion Core Complex" Journal of Virology (2003) 77(16):8801-8811.

PEP1 (SEQ ID NO: 7197), PEP2 (SEQ ID NO: 7198) and PEP3 (SEQ ID NO: 7199) sequences within the SARS spike protein have been identified.

The coronavirus spike proteins (and other similar surface viral proteins) are thought to undergo a conformational change upon receptor binding to the target cell membrane. One or more of the hydrophobic Membrane Fusion peptides are thought to become exposed and inserted into the target membrane as a result of this conformational change. The free energy released

upon subsequent refolding of the spike protein to its most stable conformation is believed to play a role in the merger of the viral and cellular membranes.

One or more SARS HR sequences, preferably HR2, or a fragment thereof may be used to inhibit viral entry and membrane fusion with a target mammalian host cell. The invention provides a method of inhibiting viral infection comprising administering a composition comprising one or more SARS HR polypeptides or a fragment thereof. Preferably, the composition comprises a SARS HR2 sequence.

In another embodiment, the invention includes a composition comprising a SARS HR1 sequence, or a fragment thereof and a SARS HR2 sequence, or a fragment thereof. The HR1 and HR2 sequences may optionally be associated together in an oligomer. The composition may comprise the intermediate domain sequence between the HR1 and HR2 domains. The use of such an intermediate sequence may facilitate oligomerization or other structural interaction between the HR regions.

HR sequences for use in the invention may be produced recombinantly by methods known in the art. The SARS HR sequences may be modified to facilitate bacterial expression. In particular, the HR sequences may be modified to facilitate transport of the recombinant protein to the surface of the bacterial host cell. For example, leader sequences to a bacterial membrane protein may be added to the N terminus of the recombinant HR sequences. HR sequences for use in the invention may alternatively be produced by chemical synthesis by methods known in the art (see below).

As discussed in more detail later in the specification, Applicants have identified structural similarities between the SARS spike protein and the surface protein of *Neisseria meningitidis*, NadA (and other similar bacterial adhesion proteins). Another means of facilitating bacterial expression of HR sequences includes the addition of the stalk and/or anchor sequences of a NadA-like protein to the C-terminus of the recombinant HR sequences. Recombinant sequences containing the bacterial anchor sequence may preferably be prepared in outer membrane vesicles (the preparation of which is discussed in more detail later in the application). Recombinant sequences missing the bacterial anchor sequences may be secreted and isolated from the supernatant.

The invention includes a polypeptide sequence comprising a first sequence and a second sequence, wherein said first sequence comprises a leader sequence for a bacterial membrane protein and wherein said second sequence comprises a HR sequence of a coronavirus. Preferably, said first sequence comprises the leader sequence for a bacterial adhesin protein. More preferably, said bacterial adhesion protein is NadA. Preferably said second sequence comprises HR1, HR2 or both. In one embodiment, the second sequence comprises HR1, HR2 and the intermediate domain sequence present in the naturally occrding spike protein. For

example, the second sequence may comprise a fragment of a coronavirus spike protein comprising the amino acids starting with the N-terminus of the HR1 region and ending with the C-terminus of the HR2 region.

The invention further includes a polypeptide sequence comprising a first, second, third and fourth sequence, wherein the first sequence comprises a leader sequence for a bacterial membrane protein; wherein said second sequence comprises a HR sequence of a coronavirus; wherein said third sequence comprises a stalk domain of a bacterial adhesion protein; and wherein said fourth sequence comprises an anchor domain of a bacterial adhesion protein. In one embodiment, the first sequence comprising the leader peptide sequence is removed. In another embodiment, the third sequence comprising the stalk domain is removed. In another embodiment, the fourth sequence comprising the anchor domain is removed.

The polypeptide sequences of the above described constructs may be linked together by means known in the art, including, for example, via glycine linkers.

Examples of constructs which may be used in such bacterial expression systems are shown in FIGURE 50. Polypeptide sequences of each of the constructs illustrated in FIGURE 50 are given as SEQ ID NOS: 7200 to 7206.

7200	Leader NadA (1-29) - HR1 (879-980) - 6Xgly - HR2 (1144-1195) - stalk+anchor NadA (88-405)
7201	Leader NadA (1-29) - HR1 (879-980) - 6Xgly - HR2 (1144-1196) - stalk NadA (88-351)
7202	Leader NadA (1-29) - HR1 - HR2 (879-1196) - stalk+anchor NadA (88-405)
7203	Leader NadA (1-29) - HR1 - HR2 (879-1196)-stalk NadA (88-351)
7204	HR1 - HR2 (879-1196)-stalk NadA (88-351)-6xhis
7205	Leader NadA (1-29) - HR1 - HR2 (879-1196)-anchor NadA (351-405)
7206	Leader NadA (1-29) - HR1 - HR2 (879-1196)

Administration of one of more of these Membrane Fusion sequences may also interfere with the ability of a coronavirus to fuse to a host cell membrane. Accordingly, the invention includes an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7197, SEQ ID NO: 7198and SEQ ID NO: 7199. The invention further includes an isolated polypeptide comprising an amino acid sequence having sequence homology to an amino acid sequence selected from the group consisting of SEQ ID NO: 7197, SEQ ID NO: 7198and SEQ ID NO: 7199.

Two or more of these SARS Membrane Fusion peptides can be combined together. The invention includes a composition comprising two SARS Membrane Fusion peptides wherein said peptides are selected from at least two of the amino acids selected from the group consisting of SEQ ID NO: 7197, SEQ ID NO: 7198and SEQ ID NO: 7199, or a sequence having sequence identity thereto.

Two or more of the SARS Membrane Fusion peptides may be linked together. Accordingly, the invention includes a polypeptide comprising a first amino acid sequence and a

second amino acid sequence, wherein said first and second amino acid sequences are selected from the group consisting of SEQ ID NO: 7197, SEQ ID NO: 7198and SEQ ID NO: 7199, or a sequence having sequence identity thereto. Preferably, said first amino acid sequence and said second amino acid sequence are different SARS Membrane Fusion peptides, *i.e.*, they are not the same.

The invention also includes a method of treating or preventing SARS virus infection comprising administering one or more of the SARS Membrane Fusion peptide compositions described above.

As discussed above, the spike protein is capable of forming trimers. The invention further includes a polypeptide comprising SEQ ID NO: 6042 in trimeric form. The invention includes a composition comprising at least polypeptides wherein each polypeptide comprises at least the alpha-helical coiled region of a SARS virus spike protein. Preferably, the spike protein comprises SEQ ID NO: 6042 or a fragment thereof.

The invention further includes a composition comprising a SARS virus spike protein or a fragment thereof wherein said protein is associated with a transmembrane and wherein said fragment comprises the alpha-helical region of the SARS virus spike protein. Preferably, the composition comprises at least three SARS virus spike proteins or a fragment thereof, wherein the fragment comprises the alpha-helical region of the SARS virus spike protein.

The invention further includes an antibody which specifically binds to a trimeric form of SARS virus spike proteins. Preferably, the spike protein comprises SEQ ID NO: 6042 or a fragment thereof. The invention includes an antibody which specifically binds to a trimeric form of SARS virus spike proteins wherein said proteins are associated with a transmembrane.

The invention further includes an antibody which specifically binds to a monomeric form of SARS virus spike protein or a fragment thereof. Preferably, the antibody specifically binds to a monomeric form of SEQ ID NO: 6042 or a fragment thereof.

The invention further includes a small molecule which interferes with or disrupts the coiling of a SARS viral spike protein trimer.

The invention further includes an attenuated SARS virus for use as a vaccine wherein said attenuated virus contains a polynucleotide insertion, deletion or substitution which does not disrupt the trimeric conformation of the SARS virus spike protein. The invention further includes an attenuated SARS virus for use as a vaccine wherein said attenuated virus contains a polynucleotide insertion, deletion or substitution which does not disrupt the alpha-helical formation of the SARS virus spike protein.

The spike protein may be recombinantly produced. In one embodiment, the spike protein is expressed in virus like particles so that the protein is attached to a cell membrane. Such attachment may facilitate presentation of immunogenic epitopes of the spike protein. Preferably,

the alpha-helical portion of the spike protein is associated with the cell membrane. Preferably, the spike proteins form a trimer within the transmembrane region of attachment.

Predicted N-glycosylation sites of SEQ ID NO: 6042 are identified below:

Position			Potential	Jury agreement	NGlyc result
29	NYTQ	SEQ ID NO: 7207	0.7751	(9/9)	+++
65	NVTG	SEQ ID NO: 7208	0.8090	(9/9)	+++
109	NKSQ	SEQ ID NO: 7209	0.6081	(7/9)	+
119	NSTN	SEQ ID NO: 7210	0.7039	(9/9)	++
158	NCTF	SEQ ID NO: 7211	0.5808	(7/9)	+
227	NITN	SEQ ID NO: 7212	0.7518	(9/9)	+++
269	NGTI	SEQ ID NO: 7213	0.6910	(9/9)	++
318	NITN	SEQ ID NO: 7214	0.6414	(9/9)	++
330	NATK	SEQ ID NO: 7215	0.6063	(8/9)	+
357	NSTF	SEQ ID NO: 7216	0.5746	(8/9)	+
589	NASS	SEQ ID NO: 7217	0.5778	(6/9)	+
602	NCTD	SEQ ID NO: 7218	0.6882	(9/9)	++
699	NFSI	SEQ ID NO: 7219	0.5357	(7/9)	+
783	NFSQ	SEQ ID NO: 7220	0.6348	(9/9)	++
1080	NGTS	SEQ ID NO: 7221	0.5806	(7/9)	+
1116	NNTV	SEQ ID NO: 7222	0.5106	(5/9)	+
1176	NESL	SEQ ID NO: 7223	0.6796	(9/9)	++

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises one or more of the glycosylation sites identified above (SEQ ID NOS: 7207-7223). The invention further includes a polynucleotide encoding one or more of the fragments identified above. This glycosylation site can be covalently attached to a saccharide. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises one or more of the glycosylation sites identified above and wherein said polypeptide is glycosylated at one or more of the sites identified above.

Predicted O-glycosylation sites are identified below:

Residue	No.	Potential	Threshold	Assignment
Thr	698	0.8922	0.7696	T
Thr	706	0.9598	0.7870	T
Thr	922	0.9141	0.7338	T
Ser	36	0.8906	0.7264	S
Ser	703	0.8412	0.7676	S

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises one or more of the O-glycosylation sites identified above. The invention further includes a polynucleotide encoding one or more of the fragments identified above. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises one or more of the O-glycosylation sites identified above and further wherein the polypeptide is covalently bonded to a saccharide at one or more of the included glycosylation sites.

The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises one or more of the N-glycosylation sites identified above and

further wherein said fragment comprises one or more of the O-glycosylation sites identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

Predicted phosphorylation sites of SEQ ID NO: 6042 are Ser-346, Tyr-195, and Tyr-723. Accordingly, the invention comprises a polypeptide comprising a fragment of SEQ ID NO: 6042 wherein said fragment comprises at least ten amino acid residues and wherein said fragment comprises one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723. In one embodiment, one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723 are phosphorylated.

Expression and functional characterization of the spike glycoprotein has been described by Xiao *et al.* (2003) *Biochem Biophys Res Comm* 312:1159-1164.

T-epitopes for SEQ ID NO: 6042 are identified in Table 16. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified as SEQ ID NOS: 8041-8280; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 8041-8280, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6040. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 6040. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 6040.

The invention includes a polynucleotide encoding SEQ ID NO: 6040 or a fragment thereof. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6040 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 6040 or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6040 or a fragment thereof.

SEQ ID NO: 6040 demonstrates functional homology with a membrane protein of coronaviruses. Predicted transmembrane helices of SEQ ID NO: 6040 are identified below:

```
Predicted Transmembrane Helices

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

        Inside to outside helices.:   3 found
              from          to    score center
          27 (  30)  48 (  45)    1138     38
         137 ( 139) 153 ( 153)     486    146

        Outside to inside helices :   3 found
              from          to    score center
          28 (  31)  45 (  45)     819     38
          71 (  73)  90 (  90)     210     81
         136 ( 142) 156 ( 156)     272    149
```

The amino acid region with the highest predicted transmembrane helical region is from amino acid position 27 to 48 of SEQ ID NO: 6040. Such transmembrane regions are often difficult to express recombinantly. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6040 wherein said fragment does not include the amino acid sequence between positions 27 to 48. The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6040 wherein said fragment does not include the amino acid sequence between positions 28 to 45. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6040 is predicted to be a hypothetical protein of the SARS virus. A prediction of the protein localization of SEQ ID NO: 6040 is set forth below. SEQ ID NO: 6040 is predicted to be located in one of the following locations: mitochondrial matrix space, microbody (peroxisome), nucleus, and mitochondrial inner membrane. SEQ ID NO: 6040 is predicted to be associated with an organelle inside an infected cell.

Accordingly, SEQ ID NO: 6040 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6040 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6040 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6040 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6040 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ

ID NO: 6040 from associating with an organelle inside of an infected cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6040.

```
PSORT --- Prediction of Protein Localization Sites
                                        version 6.4(WWW)
SEQ ID NO: 6040          163 Residues
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 0
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   9
     Peak Value of UR:   1.75
     Net Charge of CR: 1
     Discriminant Score:     -2.56
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 1.94
     Possible cleavage site: 53
>>> Seems to have no N-terminal signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
ALOM new cnt: 0 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 0  value:   1.32  threshold: -2.0
     PERIPHERAL  Likelihood =  1.32
     modified ALOM score:  -1.16
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 156
     HRSVTI
Discrimination of mitochondrial target seq.:
     notclr ( 0.88)
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 156)  from: 27  to: 44  Score:  5.0
Mitochondrial matrix?  Score:  0.36
SKL motif (signal for peroxisomal protein):
     pos: 99(163), count: 1   SKL
     SKL score (peroxisome):  0.3
Amino Acid Composition Tendency for Peroxisome:  -4.28
Peroxisomal proteins?   Status: notclr
Amino Acid Composition tendency for lysosomal proteins
     score:  0.02  Status: notclr
Modified score for lysosome: 0.152
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
     Found: pos: 132  (5) KRKR
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
nuc modified.   Score:  0.60
Nuclear Signal   Status: notclr ( 0.30)
```

```
Checking CaaX motif..
Checking N-myristoylation..
Checking CaaX motif..

----- Final Results -----
mitochondrial matrix space --- Certainty= 0.480(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.300(Affirmative) < succ>
nucleus --- Certainty= 0.300(Affirmative) < succ>
mitochondrial inner membrane --- Certainty= 0.188(Affirmative) < succ>
```

Predicted N-glycosylation sites of SEQ ID NO: 6040 are identified below.

Position	Potential	Jury agreement	NGlyc result
2 NKTG (SEQ ID NO: 7255)	0.7804	(9/9)	+++
106 NLTL (SEQ ID NO: 7256)	0.6123	(7/9)	+

Accordingly, the invention comprises a fragment of SEQ ID NO: 6040 wherein said fragment is at least ten amino acids and wherein said fragment comprises one or more of the asparagines from the amino acid positions of SEQ ID NO: 6040 selected from the group consisting of 2 and 106. The invention includes a fragment of SEQ ID NO: 6040 wherein said fragment comprises one or more amino acid sequences selected from the group consisting of SEQ ID NO: 7255 and SEQ ID NO: 7256. Preferably, the fragment comprises the amino acid sequence NKTG (SEQ ID NO: 7255).

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6040 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6040 are identified in Table 14. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 7640-7800; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 7640-7800, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus.

The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6041. SEQ ID NO: 6041 demonstrates functional homology with a portion of an ORF 1ab polyprotein. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 6041. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 6041. The invention includes a polynucleotide sequence encoding an amino acid sequence having sequence identity to SEQ ID NO: 6041. The invention includes a polynucleotide encoding a fragment of a polypeptide comprising SEQ ID NO: 6041.

The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6041 or a fragment therof. The invention includes a diagnostic kit comprising a polynucleotide encoding SEQ ID NO: 6041 or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 6041 or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6041 or a fragment thereof.

The polyproteins of coronaviruses are associated with enzymatic activity. Accordingly, SEQ ID NO: 6041 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprising SEQ ID NO: 6041 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6041 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6041 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6041 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6041 from performing enzymative activity. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6041.

Predicted transmembrane or hydrophobic regions of SEQ ID NO: 6041 are identified below. Although the polyprotein of coronaviruses is proteolytically cleaved into numerous smaller proteins, hydrophobic domains in the polyprotein are known to mediate the membrane association of the replication complex and to be able to dramatically alter the architecture of host cell membranes. Accordingly, the hydrophobic domains of the polyprotein are targets for genetic mutation to develop attenuated SARS virus vaccines. The hydrophobic domains are also

targets for small molecule inhibitors of the SARS virus. The hydrophobic domains may also be used to generate antibodies specific to those regions to treat or prevent SARS virus infection.

Possible transmembrane helices of SEQ ID NO: 6041

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

Inside to outside helices : 18 found

from		to		score	center
234	(234)	254	(250)	1046	241
256	(256)	272	(270)	252	263
319	(319)	334	(334)	227	327
503	(505)	522	(519)	405	512
613	(615)	633	(629)	619	622
677	(679)	703	(696)	467	689
849	(851)	869	(865)	229	858
1080	(1080)	1097	(1094)	306	1087
1147	(1149)	1163	(1163)	354	1156
1557	(1557)	1581	(1577)	817	1567
1954	(1954)	1971	(1971)	832	1964
2369	(2372)	2395	(2387)	300	2379
2513	(2513)	2532	(2529)	690	2522

Outside to inside helices : 14 found

from		to		score	center
239	(239)	254	(254)	924	247
239	(248)	272	(263)	468	256
311	(314)	334	(328)	267	321
499	(503)	522	(519)	485	512
617	(617)	634	(631)	425	624
849	(853)	872	(872)	572	864
1147	(1147)	1162	(1162)	765	1155
1564	(1564)	1581	(1579)	883	1572
1951	(1951)	1968	(1966)	657	1958
2513	(2522)	2539	(2537)	711	2529

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6041, wherein said fragment comprises an amino acid sequence including one or more of the hydrophobic transmembrane sequences identified above. The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6041 wherein said fragment comprises one or more of the following polypeptide sequences of SEQ ID NO: 6041: 234-254, 613-633, 1557-1581, 1954-1971, 2513-2532, 239-254, 1564-1581, 1951-1968, 2513-2539. Preferably, the fragment comprises one or more of the following polypeptide sequences of SEQ ID NO: 6041: 234-254 and 239-254. The invention also includes polynucleotides encoding each of the polypeptide fragments identified above.

The invention includes an attenuated SARS virus wherein said attenuated SARS virus contains an addition, deletion or substitution in the polynucleotides encoding for one of the hydrophobic domains identified above. The invention also includes a method for creating an attenuated SARS virus comprising mutating a SARS virus by adding, deleting or substituting the

viral genome of the SARS virus to alter the coding of one or more of the hydrophobic domains of SEQ ID NO: 6041 identified above.

The invention includes an antibody which specifically identifies one or more of the hydrophobic regions of SEQ ID NO: 6041 identified above. The invention includes a small molecule which binds to, interferes with the hydrophobicity of or otherwise disrupts one or more of the hydrophobic regions of SEQ ID NO: 6041 identified above.

Predicted N-glycosylation sites of SEQ ID NO: 6041 are identified below:

Position	Potential	Jury agreement	NGlyc result
571 NLSH (SEQ ID NO: 7257)	0.6598	(8/9)	+
835 NTSR (SEQ ID NO: 7258)	0.5762	(7/9)	+
958 NVTD (SEQ ID NO: 7259)	0.7494	(9/9)	++
1113 NISD (SEQ ID NO: 7260)	0.7259	(8/9)	+
1205 NSTL (SEQ ID NO: 7261)	0.6296	(9/9)	++
1460 NVTG (SEQ ID NO: 7262)	0.6844	(9/9)	++
1685 NHSV (SEQ ID NO: 7263)	0.5181	(5/9)	+
2029 NKTT (SEQ ID NO: 7264)	0.5423	(5/9)	+

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6041, wherein said fragment comprises one or more of the N-glycosylation sites identified above. The invention comprises a polypeptide comprising a fragment of SEQ ID NO: 6041 wherein said fragment comprises one or more of sequences SEQ ID NOS: 7257-7264. Preferably, the fragment comprises one or more of the sequences SEQ ID NOS: 7257, 7259, 7260, 7261 and 7262. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6041 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6041 are identified in Table 15. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 7801-8040; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 7801-8040, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a

CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus.

The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide sequence SEQ ID NO: 6043 or a fragment thereof. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 6043. The invention includes a polynucleotide sequence encoding the amino acid sequence of SEQ ID NO: 6043 or a fragment thereof.

Predicted transmembrane regions of SEQ ID NO: 6043 are set forth below.

```
Inside to outside helices :   4 found
      from          to    score center
  41 (  41)  56 (  56)    1789     49
  76 (  79)  99 (  99)    2142     89
 105 ( 105) 125 ( 125)    1250    115

Outside to inside helices :   3 found
      from          to    score center
  41 (  41)  59 (  56)    2053     49
  76 (  82)  98 (  96)    1580     89
 103 ( 105) 125 ( 123)    1257    115
```

The amino acid region with the highest predicted transmembrane helical region is from amino acid position 76 to 99 of SEQ ID NO: 6043. Such transmembrane regions are often difficult to express recombinantly. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6043 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. Preferably, the fragment does not include the amino acids between positions 27 to 48. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6043 is predicted to be a hypothetical protein of the SARS virus. A prediction of the protein localization of SEQ ID NO: 6043 is set forth below. SEQ ID NO: 6043 is predicted to be located in one of the following locations: mitochondrial inner membrane, plasma membrane, Golgi body, and mitochondrial intermembrane space. SEQ ID NO: 6043 may be associated with an organelle inside an infected cell.

Accordingly, SEQ ID NO: 6043 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6043 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID

NO: 6043 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6043 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6043 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6043 from associating with an organelle inside of an infected cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6043.

```
PSORT --- Prediction of Protein Localization Sites for SEQ ID NO: 6043
                                                  version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 3
     Position of the most N-terminal TMS: 40 at i=2
MTOP: membrane topology (Hartmann et al.)
     I(middle): 47   Charge diffirence(C-N):  3.5
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   12
     Peak Value of UR:   1.41
     Net Charge of CR: 0
     Discriminant Score:     -4.67
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 3.44
     Possible cleavage site: 15
>>> Seems to have no N-terminal signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
ALOM new cnt: 2 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 2  value:  -6.90  threshold: -2.0
     INTEGRAL    Likelihood = -6.90   Transmembrane   83 -   99 (  78 - 101)
     INTEGRAL    Likelihood = -5.04   Transmembrane   40 -   56 (  37 -  60)
     PERIPHERAL  Likelihood = -0.32
     modified ALOM score:   1.48
>>> Likely a Type IIIb membrane protein (Nexo Ccyt)
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 128
     MRCWLC
Discrimination of mitochondrial target seq.:
     notclr ( 0.76)
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein

*** Reasoning Step: 2

Type IIIa or IIIb is favored for ER memb. proteins
KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 128)  from: 39  to: 56  Score: 11.5
>>> Seems to have an intramitochondrial signal
Mitochondrial inner membrane?  Score:  0.59
Mitochondrial intermemb.space?  Score:  0.22
SKL motif (signal for peroxisomal protein):
     pos: 92(274), count: 1   SHL
```

```
     SKL score (peroxisome): 0.3
Amino Acid Composition Tendency for Peroxisome:   4.78
Peroxisomal proteins?   Status: positive
Amino Acid Composition tendency for lysosomal proteins
     score:  1.16  Status: notclr
Type III proteins may be localized at Golgi
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Check the Number of TMSs for typeIII (plasma memb.)
Checking N-myristoylation..

----- Final Results -----
```

mitochondrial inner membrane --- Certainty= 0.664(Affirmative) < succ>
plasma membrane --- Certainty= 0.600(Affirmative) < succ>
Golgi body --- Certainty= 0.400(Affirmative) < succ>
mitochondrial intermembrane space --- Certainty= 0.362(Affirmative) < succ>

Predicted N- and O- glycosylation sites of SEQ ID NO: 6043 are identified below.

Position	Potential	Jury agreement	NGlyc result
227 NATF (SEQ ID NO: 7265)	0.6328	(7/9)	+

Residue	No.	Potential	Threshold	Assignment
Thr	28	0.9095	0.6280	T
Thr	32	0.8740	0.6595	T
Thr	34	0.9058	0.6655	T
Thr	170	0.6816	0.6600	T
Thr	267	0.9240	0.5779	T
Thr	268	0.7313	0.5708	T
Thr	269	0.9859	0.5583	T
Thr	270	0.8023	0.5492	T
Ser	27	0.6930	0.6091	S
Ser	252	0.6457	0.5977	S

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6043, wherein said fragment comprises the N-glycosylation sites or O-glycosylation sites identified above. The invention comprises a polypeptide comprising a fragment of SEQ ID NO: 6043 wherein said fragment comprises one or more of the N-glycosylation sites or O-glycosylation sites identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6043 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6043 are identified in Table 17. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 8281-8486; (b) an amino acid sequence having sequence identity to an amino acid

sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 8281-8486, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6044. The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6044 or a sequence having sequence identity to SEQ ID NO:206. The invention includes a polynucleotide encoding SEQ ID NO: 6044.

SEQ ID NO: 6044 is identified as a hypothetical protein. Predicted hydrophobic or transmembrane regions of SEQ ID NO: 6044 are identified below:

```
Inside to outside helices :   3 found
      from          to    score center
   1 (   1)  17 (  15)    891      8
  47 (  47)  66 (  63)    221     56

Outside to inside helices :   4 found
      from          to    score center
   1 (   4)  21 (  19)    599     11
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6044 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. Preferably, the fragment does not include the amino acids between positions 1 to 19. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6044 is predicted to be a hypothetical protein of SARS virus. A prediction of the protein localization of SEQ ID NO: 6044 is set forth below. SEQ ID NO: 6044 is predicted to be located in one of the following locations: nucleus, mitochondrial matrix, lysosome (lumen),

and microbody (peroxisome). SEQ ID NO: 6044 may be associated with an organelle inside an infected cell.

Accordingly, SEQ ID NO: 6044 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6044 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6044 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6044 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6044 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6044 from associating with an organelle inside of an infected cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6044.

```
PSORT --- Prediction of Protein Localization Sites for SEQ ID NO: 6044
                                                  version 6.4(WWW)
154 Residues
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
    count: 0
McG: Examining signal sequence (McGeoch)
    Length  of  UR:   7
    Peak Value of UR:   1.06
    Net Charge of CR: 1
    Discriminant Score:     -7.97
GvH: Examining signal sequence (von Heijne)
    Signal Score (-3.5): -3.28
    Possible cleavage site: 34
>>> Seems to have no N-terminal signal seq.
Amino Acid Composition of Predicted Mature Form:
  calculated from 1
ALOM new cnt: 0 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
    count: 0  value:   1.43  threshold: -2.0
    PERIPHERAL  Likelihood =  1.43
    modified ALOM score:  -1.19
Gavel: Examining the boundary of mitochondrial targeting seq.
     motif at: 151
    FRKKQV
Discrimination of mitochondrial target seq.:
    notclr (-0.46)

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 151)  from: 46  to: 50  Score:  5.0
Mitochondrial matrix?  Score:  0.36
SKL motif (signal for peroxisomal protein):
    pos: -1(154), count: 0
Amino Acid Composition Tendency for Peroxisome:   0.61
Peroxisomal proteins?   Status: notclr
    AAC score (peroxisome): 0.149
Amino Acid Composition tendency for lysosomal proteins
```

```
     score:  0.81  Status: notclr
Modified score for lysosome: 0.231
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
     Found: pos: 134 (3) KHKK
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
     Found:  pos: 136 (3) KK VSTNLCTHSF RKKQV
Final Robbins Score (nucleus):  0.60
Checking the RNA binding motif (nucleus or cytoplasm)
nuc modified.   Score:  0.90
Nuclear Signal   Status: positive ( 0.70)
Checking CaaX motif..
Checking N-myristoylation..
Checking CaaX motif..

----- Final Results -----
nucleus --- Certainty= 0.880(Affirmative) < succ>
mitochondrial matrix space --- Certainty= 0.360(Affirmative) < succ>
lysosome (lumen) --- Certainty= 0.231(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.149(Affirmative) < succ>
```

One predicted O-glycosylation site of SEQ ID NO: 6044 is identified at residue 4:

```
Residue No.  Potential Threshold Assignment
Thr    4       0.6839   0.6484       T
```

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6044, wherein said fragment comprises the O-glycosylation site identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6044 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6044 are identified in Table 18. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 8487-8665; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 8487-8665, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS

virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide sequence comprising SEQ ID NO: 6045. The invention includes a polypeptide sequence comprising an amino acid sequence having sequence identity to SEQ ID NO: 6045. The invention includes a polypeptide sequence comprising a fragment of SEQ ID NO: 6045. The invention includes a polynucleotide sequence encoding any of these polypeptides.

SEQ ID NO: 6045 demonstrates functional homology with the envelope or small membrane protein of coronaviruses. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6045 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide encoding SEQ ID NO: 6045 or a fragment thereof. The invention includes an immunogenic composition comprising SEQ ID NO: 6045 or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6045 or a fragment thereof.

Predicted transmembrane regions of SEQ ID NO: 6045 are identified below:

```
Inside to outside helices :   1 found
      from         to    score center
  17 (  19)  33 (  33)   2881      26

Outside to inside helices :   1 found
      from         to    score center
  17 (  17)  34 (  34)   2981      27
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6045 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. Preferably, the fragment does not include the amino acids between positions 17 to 34. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides. In one embodiment, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6045 wherein said fragment does not include amino acid residues 1-34 of SEQ ID NO: 6045.

Predicted protein Localization Site of SEQ ID NO: 6045 is below.

```
PSORT --- Prediction of Protein Localization Sites for SEQ ID NO: 6045
                                                  version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1
```

```
Preliminary Calculation of ALOM (threshold: 0.5)
     count: 2
     Position of the most N-terminal TMS: 17 at i=1
MTOP: membrane topology (Hartmann et al.)
     I(middle): 24   Charge diffirence(C-N):  2.0
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   29
     Peak Value of UR:   3.40
     Net Charge of CR: -2
     Discriminant Score:     13.07
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 4.37
     Possible cleavage site: 32
... positive value of mtop ...
>>> Seems to have an uncleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
.  calculated from 1
ALOM new cnt: 1 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 1  value: -15.12  threshold: -2.0
     INTEGRAL    Likelihood =-15.12   Transmembrane   17 -  33 (   8 -  44)
     PERIPHERAL  Likelihood =  0.47
     modified ALOM score:   3.12
>>> Seems to be a Type Ib (Nexo Ccyt) membrane protein
     The cytoplasmic tail is from 34 to 76 (44 Residues)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
( 6) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 6
     Uncleavable? Ipos set to: 16
Discrimination of mitochondrial target seq.:
     notclr ( 0.19)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

> Relative position of the end of the tail: 44%
Memb.protein with uncleavable signl is often at ER
KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 16)  from: 70  to: 99  Score: 21.5
>>> Seems to have an intramitochondrial signal
SKL motif (signal for peroxisomal protein):
     pos: -1(76), count: 0
Amino Acid Composition Tendency for Peroxisome:  -4.11
Peroxisomal proteins?   Status: negative
Amino Acid Composition tendency for lysosomal proteins
     score:  0.68  Status: notclr
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Check cytoplasmic tail for typeIb (plasma memb.)
```

```
Checking the NPXY motif..
Checking the YXRF motif..
Checking N-myristoylation..

----- Final Results -----
plasma membrane --- Certainty= 0.730(Affirmative) < succ>
endoplasmic reticulum (membrane) --- Certainty= 0.640(Affirmative) < succ>
endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>
outside --- Certainty= 0.100(Affirmative) < succ>
```

Predicted N-glycosylation sites of SEQ ID NO: 6045 are identified at residues 48 and 66:

Position	Potential	Jury agreement	NGlyc result	
48 NVSL	0.6514	(9/9)	++	(SEQ ID NO: 7266)
66 NSSE	0.5880	(7/9)	+	(SEQ ID NO: 7267)

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6045, wherein said fragment comprises one or more of the N-glycosylation sites identified above. The invention comprises a polypeptide comprising a fragment of SEQ ID NO: 6045 wherein said fragment comprises one or more of the N-glycosylation sites identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 6045 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

T-epitopes for SEQ ID NO: 6045 are identified in Table 19. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 8666-8820; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 8666-8820, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide sequence comprising SEQ ID NO: 6046. The invention includes polypeptide sequences comprising an amino acid sequence having sequence identity to SEQ ID NO: 6046. The invention includes a polypeptide sequence comprising a fragment of SEQ ID NO: 6046. The invention includes a polynucleotide encoding one of these polypeptides.

SEQ ID NO: 6046 has functional homology with a matrix protein of a coronavirus. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6046 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide encoding SEQ ID NO: 6046 or a fragment thereof. The invention includes an immunogenic composition comprising SEQ ID NO: 6046 or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 6046 or a fragment thereof.

Predicted transmembrane regions of SEQ ID NO: 6046 are identified below.

```
Inside to outside helices :   3 found
      from         to     score center
  21 (  21)  38 (  36)    2412     29
  51 (  53)  69 (  69)    2645     60
  74 (  82)  96 (  96)    2464     89

Outside to inside helices :   3 found
      from         to     score center
  18 (  21)  38 (  38)    2363     28
  52 (  52)  67 (  67)    2363     60
  76 (  76)  95 (  92)    2605     84
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6046 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. Preferably, the fragment does not include the amino acids between positions selected from the group consisting of 18 to 38, 52 to 67 and 76 to 95. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

Predicted protein localization of SEQ ID NO: 6046 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                             version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 3
     Position of the most N-terminal TMS: 21 at i=1
MTOP: membrane topology (Hartmann et al.)
     I(middle): 28   Charge diffirence(C-N):  6.0
```

```
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   1
     Peak Value of UR:   3.16
     Net Charge of CR: -3
     Discriminant Score:     2.21
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 4.29
     Possible cleavage site: 39
... positive value of mtop ...
>>> Seems to have an uncleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 3  value:  -7.64  threshold:  0.5
     INTEGRAL    Likelihood = -7.64   Transmembrane   21 -  37 (  18 -  39)
     INTEGRAL    Likelihood = -7.59   Transmembrane   50 -  66 (  43 -  72)
     INTEGRAL    Likelihood = -5.04   Transmembrane   79 -  95 (  72 -  99)
     PERIPHERAL  Likelihood =  2.38
     modified ALOM score:   2.13
>>> Likely a Type IIIb membrane protein (Nexo Ccyt)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
( 2) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 2
     Uncleavable? Ipos set to: 12
Discrimination of mitochondrial target seq.:
     negative (-4.16)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

Type IIIa or IIIb is favored for ER memb. proteins
Memb.protein with uncleavable signl is often at ER
KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
SKL motif (signal for peroxisomal protein):
     pos: -1(221), count: 0
Amino Acid Composition Tendency for Peroxisome:   5.01
Peroxisomal proteins?   Status: notclr
Amino Acid Composition tendency for lysosomal proteins
     score:  2.30  Status: positive
Type III proteins may be localized at Golgi
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Check the Number of TMSs for typeIII (plasma memb.)
Checking N-myristoylation..

----- Final Results -----
endoplasmic reticulum (membrane) --- Certainty= 0.685(Affirmative) < succ>
plasma membrane --- Certainty= 0.640(Affirmative) < succ>
Golgi body --- Certainty= 0.460(Affirmative) < succ>
endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>
```

One predicted N-glycosylation sites of SEQ ID NO: 6046 is identified at residue 4:

Prediction of N-glycosylation sites

Position	Potential	Jury agreement	NGlyc result	
4 NGTI	0.8430	(9/9)	+++	(SEQ ID NO: 7268)

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6046, wherein said fragment comprises the N-glycosylation site identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention further comprises a polypeptide comprising a fragment of amino acid sequence SEQ ID NO: 6046, wherein said fragment does not include the N-glycosylation site identified above. The invention includes a polynucleotide encoding such a fragment.

A variant of SEQ ID NO: 6046 that is included within the invention is SEQ ID NO: 9963. Compared to SEQ ID NO: 6046, this sequence has Val at residue 72 instead of Ala.

T-epitopes for SEQ ID NO: 6046 are identified in Table 20. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 8821-9018; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 8821-9018, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide sequence comprising SEQ ID NO: 6047 or a fragment thereof or an amino acid sequence having sequence identity thereto. Predicted transmembrane regions of SEQ ID NO: 6047 are identified below.

```
Inside to outside helices :   2 found
       from          to    score center
   7 (  10)  29 (  27)    729      17
  21 (  24)  41 (  41)    640      34

Outside to inside helices :   2 found
       from          to    score center
   4 (   4)  22 (  19)    874      12
  22 (  24)  41 (  41)    499      31
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6047 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. Preferably, the fragment does not include the amino acids between positions selected from the group consisting of 4 to 22 and 22 to 41. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6047 is predicted to be a hypothetical protein of SARS virus. A prediction of the protein localization of SEQ ID NO: 6047 is set forth below. SEQ ID NO: 6047 is predicted to be located in one of the following locations: plasma membrane, endoplasmic reticulum, Golgi body, and microbody (peroxisome). SEQ ID NO: 6047 may be associated with an organelle inside an infected cell or with viral entry to a host cell.

Accordingly, SEQ ID NO: 6047 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6047 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6047 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6047 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6047 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6047 from associating with an organelle inside of an infected cell or interacting with a host cell membrane. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6047. Predicted protein localization of SEQ ID NO: 6047 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                          version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 1
     Position of the most N-terminal TMS: 2 at i=1
MTOP: membrane topology (Hartmann et al.)
     I(middle): 9   Charge diffirence(C-N):  0.5
McG: Examining signal sequence (McGeoch)
```

```
     Length  of  UR:   6
     Peak Value of UR:   3.08
     Net Charge of CR: 0
     Discriminant Score:      5.12
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): -4.45
     Possible cleavage site: 34
>>> Seems to have an uncleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
ALOM new cnt: 1 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 1  value:  -2.44  threshold: -2.0
     INTEGRAL    Likelihood = -2.44   Transmembrane    2 -  18 (   1 -  20)
     PERIPHERAL  Likelihood =  1.22
     modified ALOM score:   0.59
>>> Seems to be a Type II (Ncyt Cexo) membrane protein
     The cytoplasmic tail is from 1 to 1 (1 Residues)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
( 5) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 5
     Uncleavable? Ipos set to: 15
Discrimination of mitochondrial target seq.:
     notclr ( 1.48)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

Relative position of the cytoplasmic tail: 1%
     Larger value (>30%) is favared for ER memb. proteins
Memb.protein with uncleavable signl is often at ER
KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 15)  from: 64  to: 93  Score: 30.0
>>> Seems to have an intramitochondrial signal
SKL motif (signal for peroxisomal protein):
     pos: -1(63), count: 0
Amino Acid Composition Tendency for Peroxisome:   1.91
Peroxisomal proteins?   Status: notclr
     AAC score (peroxisome): 0.161
Amino Acid Composition tendency for lysosomal proteins
     score:  0.04  Status: notclr
Checking the consensus for Golgi
Checking the consensus for Golgi
Checking the cytoplasmic tail of type II (Golgi)
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Check mitochondrial signal for typeII (plasma memb.)
Type II is favored for plasma memb. proteins
Checking the NPXY motif..
Checking the YXRF motif..
```

```
Checking N-myristoylation..

----- Final Results -----
plasma membrane --- Certainty= 0.685(Affirmative) < succ>
endoplasmic reticulum (membrane) --- Certainty= 0.640(Affirmative) < succ>
Golgi body --- Certainty= 0.370(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.161(Affirmative) < succ>
```

T-epitopes for SEQ ID NO: 6047 are identified in Table 21. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 9019-9131; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 9019-9131, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6048, a fragment thereof or an amino acid sequence having sequence identity thereto. Predicted transmembrane regions of SEQ ID NO: 6048 are identified below.

```
Inside to outside helices :   2 found
      from         to    score center
   3 (   3)  18 (  18)   1857     10
 100 ( 100) 117 ( 115)   2904    107

Outside to inside helices :   2 found
      from         to    score center
   1 (   1)  15 (  15)   1299      8
 100 ( 100) 117 ( 115)   3009    107
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6048 wherein said fragment does not include one or more of the hydrophobic amino acid

sequences identified above. Preferably, the fragment does not include the amino acids between positions selected from the group consisting of 1 to 15 and 100 to 117. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6048 is predicted to be a hypothetical protein of SARS virus. A prediction of the protein localization of SEQ ID NO: 6048 is set forth below. SEQ ID NO: 6048 is predicted to be located in one of the following locations: plasma membrane, lysosome (membrane), microbody (peroxisome), and endoplasmic reticulum (membrane). SEQ ID NO: 6048 may be associated with an organelle inside an infected cell or may interact with a host cell plasma membrane during viral entry to the host cell.

Accordingly, SEQ ID NO: 6048 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6048 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6048 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6048 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6048 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6048 from associating with an organelle inside of an infected cell or prevents the polypeptide from associating with the cell membrane of a host cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6048. Predicted protein localization of SEQ ID NO: 6048 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                                   version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 2
     Position of the most N-terminal TMS: 3 at i=2
MTOP: membrane topology (Hartmann et al.)
     I(middle): 10   Charge diffirence(C-N): -2.5
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   13
     Peak Value of UR:   3.38
     Net Charge of CR: 1
     Discriminant Score:     10.02
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 2.56
     Possible cleavage site: 15
>>> Seems to have a cleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 16
ALOM new cnt: 2 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 1B
ALOM: finding transmembrane regions (Klein et al.)
     count: 1  value: -14.75  threshold: -2.0
     INTEGRAL    Likelihood =-14.75   Transmembrane  101 - 117 (  95 - 120)
     PERIPHERAL  Likelihood =  6.63
     modified ALOM score:   3.05
```

```
>>> Seems to be a Type Ia membrane protein
     The cytoplasmic tail is from 118 to 122 (5 Residues)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
(15) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 15
     Uncleavable? Ipos set to: 25
Discrimination of mitochondrial target seq.:
     notclr ( 0.73)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 25)  from: 3  to: 12  Score:  8.5
SKL motif (signal for peroxisomal protein):
     pos: -1(122), count: 0
Amino Acid Composition Tendency for Peroxisome:   2.46
     AAC not from the N-term., score modified
Peroxisomal proteins?  Status: notclr
     AAC score (peroxisome): 0.115
Amino Acid Composition tendency for lysosomal proteins
     score: -0.40  Status: negative
GY motif in the tail of typeIa? (lysosomal)
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Type Ia is favored for plasma memb. proteins
Checking the NPXY motif..
Checking the YXRF motif..
Checking N-myristoylation..
Checking GPI anchor..
>>> Seems to be GPI-anchored  (0.85)

----- Final Results -----
plasma membrane --- Certainty= 0.919(Affirmative) < succ>
lysosome (membrane) --- Certainty= 0.200(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.115(Affirmative) < succ>
endoplasmic reticulum (membrane) --- Certainty= 0.100(Affirmative) < succ>
```

T-epitopes for SEQ ID NO: 6048 are identified in Table 22. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 9132-9308; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further

comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 9132-9308, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6049, a fragment thereof or an amino acid sequence having sequence identity thereto. Predicted transmembrane or hydrophobic regions of SEQ ID NO: 6049 are identified below.

```
Inside to outside helices :   1 found
      from         to    score center
   13 (   13)  30 (  28)   3532     20

Outside to inside helices :   1 found
      from         to    score center
    9 (   11)  29 (  26)   3395     19
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6049 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6049 is predicted to be a hypothetical protein of SARS virus. A prediction of the protein localization of SEQ ID NO: 6049 is set forth below. SEQ ID NO: 6049 is predicted to be located in one of the following locations: outside, microbody (peroxisome), endoplasmic reticulum (membrane) and endoplasmic reticulum (lumen). The highest ranking indicates that SEQ ID NO: 6049 is located on the outside of a cell. Accordingly, SEQ ID NO: 6049 may be a surface exposed protein.

Accordingly, SEQ ID NO: 6049 may be used in an immunogenic composition to raise an immune response against the SARS virus. It also may be used to generate antibodies specific to the SARS virus. Such antibodies may be used in a method of treatment or prevention of a SARS virus infection. Such antibodies may further be used in a diagnostic test to identify the presence or absence of SARS virus in a biological sample.

The invention includes a polypeptide comprises SEQ ID NO: 6049 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6049 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6049 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6049 in a host cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6049. Predicted protein localization of SEQ ID NO: 6049 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                              version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 1
     Position of the most N-terminal TMS: 11 at i=1
MTOP: membrane topology (Hartmann et al.)
     I(middle): 18   Charge diffirence(C-N): -2.0
McG: Examining signal sequence (McGeoch)
     Length of  UR:   24
     Peak Value of UR:   3.69
     Net Charge of CR: -2
     Discriminant Score:     13.56
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): 0.52
     Possible cleavage site: 25
>>> Seems to have a cleavable N-term signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 26
ALOM new cnt: 1 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 1B
ALOM: finding transmembrane regions (Klein et al.)
     count: 0  value:  14.80  threshold: -2.0
     PERIPHERAL  Likelihood = 14.80
     modified ALOM score:  -3.86
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway
( 2) or uncleavable?
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 2
     Uncleavable? Ipos set to: 12
Discrimination of mitochondrial target seq.:
     notclr ( 1.42)
Rule: vesicular pathway
Rule: vesicular pathway
Rule: vesicular pathway

*** Reasoning Step: 2

KDEL   Count: 0
Number of Potential N-glycosylation Sites: 0
Out: score 0.800
Checking apolar signal for intramitochondrial sorting
   (Gavel position 12)  from: 44  to: 73  Score: 30.0
>>> Seems to have an intramitochondrial signal
SKL motif (signal for peroxisomal protein):
     pos: -1(44), count: 0
```

```
Amino Acid Composition Tendency for Peroxisome:   9.47
     AAC not from the N-term., score modified
Peroxisomal proteins?   Status: notclr
     AAC score (peroxisome): 0.320
Amino Acid Composition tendency for lysosomal proteins
     score: -6.47  Status: negative
Number of NX(S/T) motif: 0
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Checking CaaX motif..
Checking N-myristoylation..
Checking CaaX motif..

----- Final Results -----
outside --- Certainty= 0.820(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.320(Affirmative) < succ>
endoplasmic reticulum (membrane) --- Certainty= 0.100(Affirmative) < succ>
endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>
```

T-epitopes for SEQ ID NO: 6049 are identified in Table 23. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 9309-9437; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 9309-9437, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide comprising SEQ ID NO: 6050 or a fragment thereof or an amino acid sequence having sequence identity thereto. Predicted transmembrane or hydrophobic regions are identified below.

```
Inside to outside helices :   1 found
      from         to    score center
  13 (  15)  32 (  30)    558     23

Outside to inside helices :   1 found
      from         to    score center
  16 (  16)  30 (  30)    364     23
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6050 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

SEQ ID NO: 6050 is predicted to be a hypothetical protein of SARS virus. A prediction of the protein localization of SEQ ID NO: 6050 is set forth below. SEQ ID NO: 6050 is predicted to be located in one of the following locations: lysosome (lumen), mitochondrial matrix space, mitochondrial inner membrane, and mitochondrial intermembrane space. SEQ ID NO: 6050 may be associated with an organelle inside an infected cell during the viral replication cycle.

Accordingly, SEQ ID NO: 6050 is a target for screening of chemical inhibitors to the SARS virus. The invention includes a polypeptide comprises SEQ ID NO: 6050 or a fragment thereof. The invention includes a polynucleotide encoding the polypeptide sequence of SEQ ID NO: 6050 or a fragment thereof. The invention includes a method of screening SEQ ID NO: 6050 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6050 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6050 from associating with an organelle inside of an infected cell or prevents the polypeptide from associating with the cell membrane of a host cell. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6050. Predicted protein localization of SEQ ID NO: 6050 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                                version 6.4(WWW)
MYSEQ            84 Residues
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 0
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   3
     Peak Value of UR:   1.46
     Net Charge of CR: 2
     Discriminant Score:     -5.73
GvH: Examining signal sequence (von Heijne)
```

```
     Signal Score (-3.5): -0.12
     Possible cleavage site: 29
>>> Seems to have no N-terminal signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
ALOM new cnt: 0 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 0  value:   8.43  threshold: -2.0
     PERIPHERAL  Likelihood =  8.43
     modified ALOM score:  -2.59
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 61
     ARCWYL
Discrimination of mitochondrial target seq.:
     positive ( 1.66)
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein
Rule: mitochondrial protein

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
  (Gavel position 61)  from: 52  to: 58  Score:  6.0
Mitochondrial matrix?  Score:  0.38
SKL motif (signal for peroxisomal protein):
     pos: -1(84), count: 0
Amino Acid Composition Tendency for Peroxisome:   1.47
Peroxisomal proteins?   Status: notclr
     AAC score (peroxisome): 0.263
Amino Acid Composition tendency for lysosomal proteins
     score:  2.86  Status: positive
Modified score for lysosome: 0.850
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
Checking the RNA binding motif (nucleus or cytoplasm)
Nuclear Signal   Status: negative ( 0.00)
Checking CaaX motif..
Checking N-myristoylation..
Checking CaaX motif..

----- Final Results -----
lysosome (lumen) --- Certainty= 0.850(Affirmative) < succ>
mitochondrial matrix space --- Certainty= 0.544(Affirmative) < succ>
mitochondrial inner membrane --- Certainty= 0.266(Affirmative) < succ>
mitochondrial intermembrane space --- Certainty= 0.266(Affirmative) < succ>
```

One predicted N-glycosylation sites of SEQ ID NO: 6050 is identified at residue 43:

```
Position  Potential   Jury     NGlyc
                    agreement result
  43 NVTI   0.6713     (9/9)    ++        (SEQ ID NO: 7269)
```

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6050 wherein said fragment comprises the N-glycosylation site

identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention further comprises a polypeptide comprising a fragment of amino acid sequence SEQ ID NO: 6050 wherein said fragment does not include the N-glycosylation site identified above. The invention includes a polynucleotide encoding such a fragment.

T-epitopes for SEQ ID NO: 6050 are identified in Table 24. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 9438-9538; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 9438-9538, or a polynucleotide encoding such a polypeptide.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a polypeptide sequence comprising SEQ ID NO: 6051 or a fragment thereof or an amino acid sequence having sequence identity thereto. The invention includes a polypeptide sequence comprising SEQ ID NO: 6052 or a fragment thereof or an amino acid sequence having sequence identity thereto.

SEQ ID NO: 6051 and SEQ ID NO: 6052 demonstrate functional homology with a nucleocapsid protein of a coronavirus. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 6051, SEQ ID NO: 6052 or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide encoding SEQ ID NO: 6051, SEQ ID NO: 6052 or a fragment thereof. The invention includes an immunogenic composition comprising SEQ ID NO: 6051, SEQ ID NO: 6052 or a fragment thereof. The invention includes

an antibody which recognizes a polypeptide comprising SEQ ID NO: 6051, SEQ ID NO: 6052 or a fragment thereof.

SEQ ID NO: 6051 is predicted to be phosphorylated at Ser-79; Thr-92; Ser-106; Thr-116; Thr-142; Ser-184; Ser-188; Ser-202; Ser-236; Thr-248; Ser-251; Ser-256; Thr-377. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6051 wherein said fragment includes one or more of the amino acid residues of SEQ ID NO: 6051 selected from the group consisting of Ser-79; Thr-92; Ser-106; Thr-116; Thr-142; Ser-184; Ser-188; Ser-202; Ser-236; Thr-248; Ser-251; Ser-256; Thr-377. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 6051 wherein said fragment does not include one or more of the amino acid residues of SEQ ID NO: 6051 selected from the group consisting of Ser-79; Thr-92; Ser-106; Thr-116; Thr-142; Ser-184; Ser-188; Ser-202; Ser-236; Thr-248; Ser-251; Ser-256; Thr-377. Two further useful fragments of the N protein (*e.g.* for immunoassay) are SEQ ID NOS: 9783 & 9784, which are lysine-rich and can be used to distinguish the SARS virus from other coronaviruses.

Predicted transmembrane regions of SEQ ID NO: 6051 are identified below.

```
Inside to outside helices :   1 found
      from         to    score center
 304 ( 304) 323 ( 319)    495    312

Outside to inside helices :   2 found
      from         to    score center
304 ( 304) 319 ( 319)    597    312
```

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 6051 wherein said fragment does not include one or more of the hydrophobic amino acid sequences identified above. The invention also includes a polynucleotide sequence encoding any of the above-identified polypeptides.

Predicted protein localization of SEQ ID NO: 6051 is set forth below. SEQ ID NO: 6051 is predicted to be localized near the nucleus, lysosome (lumen), mitochondrial matrix space, and microbody (peroxisome). The highest ranking is for localization near the nucleus. Coronavirus nucleocapsid proteins are known to bind to viral RNA. Coronavirus nucleocapsid proteins are also thought to be important for cell mediated immunity. Accordingly, the invention includes a polynucleotide comprising SEQ ID NO: 6051. The invention further includes a viral vector or particle suitable for in vivo delivery of the polynucleotide sequence comprising a SARS virus nuceocapsid polynucleotide sequence or a fragment thereof. In one embodiment, the polynucleotide comprises SEQ ID NO: 6051 or a fragment thereof. The invention further includes a method for eliciting a cell mediated immune response comprising delivering a polynucleotide encoding a SARS virus nucleocapsid protein or a fragment thereof to a mammal. In one embodiment, the polynucleotide comprising SEQ ID NO: 6051 or a fragment thereof.

The invention further includes a method of screening SEQ ID NO: 6051 for an inhibitor. The invention includes the recombinant expression of SEQ ID NO: 6051 in a host cell. The invention includes a small molecule which prevents the polypeptide of SEQ ID NO: 6051 from binding to SARS virus RNA during viral replication. The invention includes a fusion protein wherein said fusion protein comprises SEQ ID NO: 6051. Predicted protein localization of SEQ ID NO: 6051 is set forth below.

```
PSORT --- Prediction of Protein Localization Sites
                                              version 6.4(WWW)
Species classification: 4

*** Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)
     count: 0
McG: Examining signal sequence (McGeoch)
     Length  of  UR:   3
     Peak Value of UR:   0.19
     Net Charge of CR: 0
     Discriminant Score:    -15.98
GvH: Examining signal sequence (von Heijne)
     Signal Score (-3.5): -6.36
     Possible cleavage site: 58
>>> Seems to have no N-terminal signal seq.
Amino Acid Composition of Predicted Mature Form:
   calculated from 1
ALOM new cnt: 0 ** thrshld changed to -2
Cleavable signal was detected in ALOM?: 0B
ALOM: finding transmembrane regions (Klein et al.)
     count: 0  value:   5.04  threshold: -2.0
     PERIPHERAL  Likelihood =  5.04
     modified ALOM score:  -1.91
Gavel: Examining the boundary of mitochondrial targeting seq.
      motif at: 17
     PRITFG
Discrimination of mitochondrial target seq.:
     negative (-3.97)

*** Reasoning Step: 2

KDEL   Count: 0
Checking apolar signal for intramitochondrial sorting
Mitochondrial matrix?  Score:  0.10
SKL motif (signal for peroxisomal protein):
     pos: -1(399), count: 0
Amino Acid Composition Tendency for Peroxisome:   0.04
Peroxisomal proteins?   Status: notclr
     AAC score (peroxisome): 0.072
Amino Acid Composition tendency for lysosomal proteins
     score:  0.96  Status: notclr
Modified score for lysosome: 0.246
Checking the amount of Basic Residues (nucleus)
Checking the 4 residue pattern for Nuclear Targeting
     Found: pos: 256 (4) KKPR
     Found: pos: 372 (5) KKKK
Checking the 7 residue pattern for Nuclear Targeting
Checking the Robbins & Dingwall consensus (nucleus)
     Found:  pos: 372 (3) KK KKTDEAQPLP QRQKK
```

```
    Found:  pos: 373 (3) KK KTDEAQPLPQ RQKKQ
Final Robbins Score (nucleus):  0.80
Checking the RNA binding motif (nucleus or cytoplasm)
nuc modified.   Score:  0.90
Nuclear Signal   Status: positive ( 0.90)
Checking CaaX motif..
Checking N-myristoylation..
Checking CaaX motif..

----- Final Results -----
nucleus --- Certainty= 0.980(Affirmative) < succ>
lysosome (lumen) --- Certainty= 0.246(Affirmative) < succ>
mitochondrial matrix space --- Certainty= 0.100(Affirmative) < succ>
microbody (peroxisome) --- Certainty= 0.072(Affirmative) < succ>
```

Predicted N-glycosylation sites of SEQ ID NO: 6051 are identified below.

Position	Potential	Jury agreement	NGlyc result	
48 NNTA	0.6879	(9/9)	++	(SEQ ID NO: 7270)
270 NVTQ	0.7684	(9/9)	+++	(SEQ ID NO: 7271)

Residue No.	Potential	Threshold	Assignment
Thr 166	0.8547	0.6439	T
Thr 367	0.5575	0.5403	T
Thr 394	0.8217	0.5821	T

Accordingly, the invention comprises a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO: 6051 wherein said fragment comprises one or more of the N-glycosylation sites identified above. The invention further comprises a polynucleotide encoding one or more of the polypeptides identified above.

The invention further comprises a polypeptide comprising a fragment of amino acid sequence SEQ ID NO: 6051 wherein said fragment does not include one or more of the N-glycosylation sites identified above. The invention includes a polynucleotide encoding such a fragment.

T-epitopes for SEQ ID NO: 6052 are identified in Table 25. The invention includes a polypeptide for use as an antigen, wherein the polypeptide comprises: (a) an amino acid sequence selected from the group consisting of the T-epitope sequences identified in SEQ ID NOS: 9539-9752; (b) an amino acid sequence having sequence identity to an amino acid sequence of (a). The invention further comprising a polynucleotide sequence encoding the polypeptides of (a) or (b). The invention further comprising a method of expression or delivery of such polynucleotides through viral vectors and/or viral particles. The invention further comprises a polypeptide comprising two or more of the T-epitope sequences identified in SEQ ID NOS: 9539-9752, or a polynucleotide encoding such a polypeptide.

A variant of SEQ ID NO: 6052 that is included within the invention is SEQ ID NO: 9964. Compared to SEQ ID NO: 6052, this sequence has Ile at residue 54 instead of Thr.

The use as an antigen is preferably a use: (1) as a T-cell antigen; (2) for generating a complex between a class I MHC protein (*e.g.* a class I HLA) and a fragment of said antigen; (3) as an antigen for raising a cell-mediated immune response; and/or (4) as an antigen for raising a CTL response. The use preferably protects or treats disease and/or infection caused by a SARS virus. The invention provides the use of a polypeptide in the manufacture of a medicament for immunising a mammal (typically a human) against SARS viral infection wherein the polypeptide is as defined above.

The invention provides a method of raising an immune response in a mammal (typically a human), comprising the step of administering to the mammal a polypeptide as defined above, wherein said immune response is a cell-mediated immune response and, preferably, a CTL response. The immune response is preferably protective or therapeutic.

The invention includes a composition comprising a SARS virus nucleocapsid protein or a fragment thereof and further comprising a SARS virus membrane protein or a fragment thereof. The composition may further comprising one or more adjuvants discussed below.

The invention further includes a composition comprising a polypeptide comprising SEQ ID NO: 6051 or a fragment thereof or a sequence having sequence identity thereto and further comprising a polypeptide comprising SEQ ID NO: 6040, or a fragment thereof or a sequence having sequence identity thereto. Such composition may be used, for instance, in a vaccine. Such composition may further comprise one or more adjuvants discussed below.

The invention includes a composition comprising a SARS virus nucleocapsid protein or a fragment thereof and a SARS virus spike protein or a fragment thereof. In one embodiment the nucleocapsid protein comprises a polypeptide sequence comprising SEQ ID NO: 6051 or a fragment thereof or a sequence having sequence identity thereto. In one embodiment, the spike protein comprises a polynucleotide comprising SEQ ID NO: 6042 or a fragment thereof or a sequence having sequence identity thereto. The composition may further comprise one or more of the adjuvants discussed below.

The invention further includes a composition comprising antibodies specific to a SARS virus nucleocapsid protein and comprising antibodies specific to a SARS virus spike protein. In one embodiment the antibody is specific to a nucleocapsid protein comprises a polypeptide sequence comprising SEQ ID NO: 6051 or a fragment thereof or a sequence having sequence identity thereto. In one embodiment, the antibody is is specific to a spike protein comprises a polynucleotide comprising SEQ ID NO: 6042 or a fragment thereof or a sequence having sequence identity thereto.

The invention further includes polynucleotide sequences, and fragments thereof, of a SARS virus which are conserved among coronaviruses, and polypeptides encoded thereby. Such conserved sequences can be identified in the alignments shown in FIGURE 7. Such conserved

sequences may be used in the vaccines of the invention or in the diagnostic reagents, kits and methods of the invention.

The invention further includes polynucleotide sequences, and fragments thereof, of a SARS virus which are specific to SARS virus and not shared with coronaviruses. Such SARS specific sequences are also identified as SEQ ID NOS: 6040, 6043, 6044, 6047, 6048, 6049 and 6050. Such SARS specific sequences may be used in the vaccines of the invention or in the diagnostic reagents, kits and methods of the invention.

The invention also includes polynucleotide sequences which can be used as probes or primers for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified inSEQ ID NOS: 6076-6265 (Table 5). The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 6076-6265.

The invention also includes polynucleotide sequences which can be used as probes or primers for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in SEQ ID NOS: 6266-6343 (Table 6). The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 6266-6343.

The invention also includes polynucleotide sequences which can be used as probes or primers for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in SEQ ID NOS: 6344-6392 (Table 7). The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 6344-6392..

The invention also includes polynucleotide sequences which can be used as probes or primers for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in SEQ ID NOS: 6393-6559 (Tables 8 & 9). The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 6393-6559.

The invention also includes polynucleotide sequences which can be used as probes or primers for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer and probe sequences identified in SEQ ID NOS: 6560-6568. The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in SEQ ID NOS: 6560-6568.

The invention includes a polypeptide sequence comprising any one of even-numbered SEQ ID NOS: 7272-7290, or a fragment thereof, or a sequence having sequence identity thereto. The invention further includes a polynucleotide sequence encoding any one of even-numbered SEQ ID NOS: 7272-7290, or a fragment thereof, or a sequence having sequence identity thereto. Examples of such polynucleotide sequences are odd-numbered SEQ ID NOS: 7273-7291.

The invention includes a polynucleotide sequence comprising an intergenic sequence which is common to each open reading frame of the SARS virus. The SARS virus is thought to use this sequence to signal translation of the open reading frame. The intergenic sequence comprises a 10mer **SEQ ID NO: 7292**, or optionally a hexamer **SEQ ID NO: 7293**. When the virus transcribes its positive (+) RNA strand to (-) RNA strand, the virus replicating structure uses the (-) strand template to transcribe nucleotides at the 5' end prior to the first intergenic sequence, followed by the intergenic sequence, followed by the selected open reading frame. The virus then creates multiple mRNAs comprising the 5' end, the intergenic sequence and coding sequence. For more details on Nidovriales replication (including Coronavirus) see *e.g.*, Ziebuhr *et al.*, "Virus-encoded proteinases and proteolytic processing in the Nidovirales", *Journal of General Virology* 81:853-879 (2000), incorporated herein by reference in its entirety.

The invention comprising a polynucleotide sequence comprising SEQ ID NO: 7292 or the complement thereof. The invention comprising a polynucleotide sequence comprising SEQ ID NO: 7293 or the complement thereof. The invention further comprises a polynucleotide sequence comprising nucleotides from the 5' end of the SARS viral genome, or its reverse complement, and further comprising an intergenic sequence or its reverse complement. The polynucleotide may further comprise one or more of the SARS virus open reading frames. Examples of polynucleotide sequences comprising nucleotides from the 5' end of the SARS virus genome followed by the intergenic sequence are SEQ ID NOS: 7294-7301.

The invention includes a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301, or a fragment thereof, or a sequence having sequence identity

thereto. In one embodiment, the polynucleotide does not consist entirely of a known SARS virus sequence.

The SARS virus intergenic sequence can be used to create a RNAi molecule. Such a SARS virus specific RNAi molecule can be used to treat SARS virus infection. The invention includes a RNAi molecule comprising a double stranded RNA molecule wherein one RNA strand comprises a sequence selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301, or a fragment thereof. Preferably, said RNA strand comprises a sequence selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the other RNA strand comprises the reverse complement of the first strand or a polynucleotide sequence which hybridizes to the first strand.

The invention includes the use of RNAi in a method of treatment for SARS virus infection comprising administering to a mammal an effective amount of the si RNA molecule. Preferably, the RNAi molecule comprises the molecule described above. Further discussion of the RNAi applications of the intergenic sequence is included in section IV of the specification below.

The invention also includes the use of a SARS virus antisense nucleotide sequence, preferably antisense directed to the SARS virus intergenic sequence. Such an antisense sequence may be used in the treatment of a subject infected with the SARS virus. The antisense of the SARS virus intergenic sequence can be designed to bind to the SARS viral polynucleotides to block access of the viral replication machinery to the intergenic sequence. Such an antisense sequence may also be used to identify the presence or absence of a SARS virus in a biological sample. The antisence can itself be labeled or the antisense associated with viral polynucleotides can be detected by means known in the art.

Antisense nucleic acids are designed to specifically bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids, with an arrest of DNA replication, reverse transcription or messenger RNA translation. Antisense polynucleotides based on a selected sequence can interfere with expression of the corresponding gene. Antisense polynucleotides will bind and/or interfere with the translation of the corresponding mRNA.

The invention also includes the use of the intergenic region with a ribozyme.

Trans-cleaving catalytic RNAs (ribozymes) are RNA molecules possessing endoribonuclease activity. Ribozymes are specifically designed for a particular target, and the target message must contain a specific nucleotide sequence. They are engineered to cleave any RNA species site-specifically in the background of cellular RNA. The cleavage event renders the mRNA unstable and prevents protein expression. Importantly, ribozymes can be used to inhibit

expression of a gene of unknown function for the purpose of determining its function in an in vitro or in vivo context, by detecting the phenotypic effect.

One commonly used ribozyme motif is the hammerhead, for which the substrate sequence requirements are minimal. Design of the hammerhead ribozyme is disclosed in Usman *et al.*, *Current Opin. Struct. Biol.* (1996) 6:527-533. Usman also discusses the therapeutic uses of ribozymes. Ribozymes can also be prepared and used as described in Long *et al.*, FASEB J. (1993) 7:25; Symons, *Ann. Rev. Biochem.* (1992) 61:641; Perrotta *et al.*, Biochem. (1992) 31:16-17; Ojwang *et al.*, *Proc. Natl. Acad. Sci.* (USA) (1992) 89:10802-10806; and US Patent 5,254,678. Ribozyme cleavage of HIV-I RNA is described in US Patent 5,144,019; methods of cleaving RNA using ribozymes is described in US Patent 5,116,742; and methods for increasing the specificity of ribozymes are described in US Patent 5,225,337 and Koizumi *et al.*, *Nucleic Acid Res.* (1989) 17:7059-7071. Preparation and use of ribozyme fragments in a hammerhead structure are also described by Koizumi *et al.*, *Nucleic Acids Res.* (1989) 17:7059-7071. Preparation and use of ribozyme fragments in a hairpin structure are described by Chowrira & Burke, *Nucleic Acids Res.* (1992) 20:2835. Ribozymes can also be made by rolling transcription as described in Daubendiek & Kool, *Nat. Biotechnol.* (1997) 15(3):273-277.

The hybridizing region of the ribozyme may be modified or may be prepared as a branched structure as described in Horn & Urdea, *Nucleic Acids Res.* (1989) 17:6959-67. The basic structure of the ribozymes may also be chemically altered in ways familiar to those skilled in the art, and chemically synthesized ribozymes can be administered as synthetic oligonucleotide derivatives modified by monomeric units. In a therapeutic context, liposome mediated delivery of ribozymes improves cellular uptake, as described in Birikh *et al.*, *Eur. J. Biochem.* (1997) 245:1-16.

Therapeutic and functional genomic applications of ribozymes proceed beginning with knowledge of a portion of the coding sequence of the gene to be inhibited. In the present invention, the target sequence preferably comprises the intergeneic sequence of the SARS virus. Preferably, the sequence is selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. A target cleavage site is selected in the target sequence, and a ribozyme is constructed based on the 5' and 3' nucleotide sequences that flank the cleavage site. Preferably, the 5' nucleotide sequence includes the 5' untranslated region of the SARS virus. The ribozyme may then further be constructed from one or more of the polynucleotide sequences selected from the group consisting of SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301.

Antisense treatment of HIV infection is described in the following references, each of which is incorporated herein by reference in their entirety. (antisense RNA complementary to the mRNA of gag, tat, rev, env) (Sezakiel *et al.*, 1991, J. Virol. 65:468-472; Chatterjee *et al.*,

1992, Science 258:1485-1488; Rhodes *et al.*, 1990, J. Gen. Virol. 71:1965. Rhodes *et al.*, 1991, AIDS 5:145-151; Sezakiel *et al.*, 1992, J. Virol. 66:5576-5581; Joshi *et al.*, 1991, J. Virol. 65:5524-5530).

The invention includes the use of decoy RNA to disrupt the SARS virus replication and life cycle. Methods of making and using such decoy RNA for treatment of a viral infection are known in the art. The invention includes delivery of genes encoding, for example, the SARS virus intergenic sequence, to infected cells. Preferably, the sequence comprises one or more of the sequences selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301. Preferably, the sequence comprises one or more of the sequences selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the sequence comprises SEQ ID NO: 7293.

In the present invention, delivery of intergenic sequence which is not linked to the SARS virus open reading frames disrupts the translation process of the viral RNA and decreases the production of vial proteins. Similar methods of treatment for HIV viral infection have been described. The following references discuss the use of decoy RNA of HIV TAR or RRE for treatment of HIV infection. Each of these references is incorporated herein by reference in their entirety. (Sullenger *et al.*, 1990, Cell 63:601-608; Sullenger *et al.*, 1991, J. Virol. 65:6811-6816; Lisziewicz *et al.*, 1993, New Biol. 3:82-89; Lee *et al.*, 1994, J. Virol. 68:8254-8264), ribozymes (Sarver *et al.*, 1990, Science 247:1222-1225; Wecrasinghe *et al.*, 1991, J. Virol. 65:5531-5534; Dropulic *et al.*, 1992, J. Virol. 66:1432-1441; Ojwang *et al.*, 1992, Proc. Natl. Acad. Sci. USA. 89:10802-10806; Yu *et al.*, 1993, Proc. Natl. Acad. Sci. USA. 90:6340-6344; Yu *et al.*, 1995, Proc. Natl. Acad. Sci. USA. 92:699-703; Yamada *et al.*, 1994, Gene Therapy 1:38-45).

The invention includes the use of the SARS virus intergenic sequence in diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. Such diagnostic reagents, kits, and methods are further discussed in Section II of the specification.

The invention includes a pair of primers for amplifying a SARS polynucleotide sequence comprising (i) a first primer comprising a sequence which is substantially identical to a portion of a sequence selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301 and (ii) a second primer comprising a sequence which is substantially complementary to a portion of a sequence selected from the group consisting of the sequence SEQ ID NO: 1and the sequence SEQ ID NO: 2, such that the primer pair (i) and (ii) defines a template sequence within a sequence from the group consisting of the sequence SEQ ID NO: 1and the sequence SEQ ID NO: 2. Preferably, the (i)

first primer comprises a sequence which is substantially identical to a portion of a sequence selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the (i) first primer comprises a sequence which is substantially identical to a portion of the sequence of SEQ ID NO: 7293. The amplicon defined by said first and second primers is preferably between 50 and 250 nucleotides in length. The primers may optionally be labeled to facilitate their detection. Methods and compositions for use in labeling primers are discussed further in the application in Section III.

The invention further includes a pair of primers for amplifying a SARS polynucleotide sequence comprising (i) a first primer comprising a sequence which is substantially identical to a portion of the complement of a portion of a sequence selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301 and (ii) a second primer comprising a sequence which is substantially complementary to a portion of the complement of a sequence selected from the group consisting of the sequence SEQ ID NO: 1and the sequence SEQ ID NO: 2, such that the primer pair defines a template sequence within a sequence selected from the group consisting of the sequence SEQ ID NO: 1 and the sequence SEQ ID NO: 2. The amplicon defined by said first and second primers is preferably between 50 and 250 nucleotides in length. The primers may optionally be labeled to facilitate their detection.. Methods and compositions for use in labeling primers are discussed further in the application in Section III.

The invention includes a kit comprising (i) a first primer comprising a sequence which is substantially identical to a portion of a sequence selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301 and (ii) a second primer comprising a sequence which is substantially complementary to a portion of a sequence selected from the group consisting of the sequence SEQ ID NO: 1 and the sequence SEQ ID NO: 2, such that the primer pair (i) and (ii) defines a template sequence within a sequence from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2. Preferably, the (i) first primer comprises a sequence which is substantially identical to a portion of a sequence selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the (i) first primer comprises a sequence which is substantially identical to a portion of the sequence of SEQ ID NO: 7293. The primers may optionally be labeled to facilitate their detection. Methods and compositions for use in labeling primers are discussed further in the application in Section III.

Other preferred kits comprise (i) a first primer comprising a sequence which is substantially identical to a portion of the complement of a portion of a sequence selected from

the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301 and (ii) a second primer comprising a sequence which is substantially complementary to a portion of the complement of a sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2, such that the primer pair defines a template sequence within a sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

The invention further includes an attenuated SARS virus for use as a vaccine wherein the intergenic region has been mutated to reduce expression of the viral structural or nonstructural proteins. The attenuated SARS virus may comprises one or more additions, deletions or insertion in one or more of the intergenic regions of the viral genome. Preferably, the attenuated SARS virus comprises an addition, deletion or insertion in one or more occurrences of the sequence selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the addition, deletion or insertion occurs in one or more occurrences of SEQ ID NO: 7293.

The invention further comprises a small molecule which inhibits binding or association of the SARS viral replication machinery, such as a ribonucleoprotein, with the intergenic region of the viral genome. Preferably, the small molecule inhibits binding or association of the SARS viral machinery with a sequence selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Preferably, the small molecule inhbiits binding or association of the SARS viral machinery with SEQ ID NO: 7293. The invention further includes a method of screening for a small molecule for treatment of SARS viral infection comprising using an assay to identify a small molecule which interferes with the association of the SARS viral replication machinery with the intergenic region of the SARS viral genome.

The invention further provides a novel SARS polynucleotide sequence SEQ ID NO: 9968. All six reading frames of this 690mer sequence are shown in Figure 113. The constituent amino acid sequences from Figure 113, having at least 4 amino acids, are listed as SEQ ID NOS: 9969 to 10032.

Accordingly the invention includes a polynucleotide sequence comprising SEQ ID NO: 9968. It also provides polynucleotide sequences having sequence identity to SEQ ID NO: 9968. The degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 70%, 80%, 85%, 88%, 90%, 92%, 95%, 99% or more).

The invention includes an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO: 9968, including the amino acid sequences selected from the group consisting of SEQ ID NO^{S}: 9969 to 10032. Preferably, the amino acid sequence comprises SEQ ID NO: 9997 or comprises SEQ ID NO: 9998.

The invention also provides amino acid sequences having sequence identity to an amino acid sequence encoded by SEQ ID NO: 9968. The invention provides amino acids having sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO^{S}: 9969 to 10032. The degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 85%, 88%, 90%, 92%, 95%, 99% or more).

A portion of SEQ ID NO: 9968 matches with approximately 98% identity to a previously published SARS polynucleotide sequence, commonly referred to as "BNI-1" (SEQ ID NO: 10033). BNI-1 was sequenced at Bernhard Nocht Institute for Tropical Medicine, National Reference Center for Tropical Infectious Diseases in Hamburg, Germany. The BNI-1 sequence was published on the WHO website on April 4, 2003 at *http://www.who.int/csr/sars/primers/en* and in Dorsten *et al.*, "Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome", *New England Journal of Medicine*, published online at *http://www.nejm.org* on April 10, 2003. Both references are incorporated herein by reference in their entirety. The six reading frames of this 302mer sequence are shown in Figure 114 (see also Figure 129). The constituent amino acid sequences from Figure 114, having at least 4 amino acids, are listed as SEQ ID NO^{S}: 10034 to 10065. An alignment of SEQ ID NO: 10034 with SEQ ID NO: 9997 is shown in Figure 130.

The invention provides for polynucleotide sequences comprising fragments of SEQ ID NO: 9968. In one embodiment, the fragment does not consist entirely of SEQ ID NO: 10033 or of a known coronavirus.

The invention provides for amino acid sequences comprising fragments of an amino acid sequence encoded by SEQ ID NO: 9968. In one embodiment, the fragment does not consist entirely of an amino acid sequence encoded by SEQ ID NO: 10033 or a known coronavirus.

The invention provides for amino acids comprising fragments of an amino acid sequence selected from the group consisting of SEQ ID NO^{S}: 9969 to 10032. In one embodiment, the fragment does not consist entirely of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO: 10033 or a known coronavirus.

Approximately 100 nucleotides at the 5' end of SEQ ID NO: 9968 do not match any portion of the BNI-1 polynucleotide sequence (SEQ ID NO: 10033). This unmatched portion is set forth as SEQ ID NO: 10066. The invention thus further provides a polynucleotide comprising the sequence comprising SEQ ID NO: 10066, polynucleotide sequences having sequence identity to SEQ ID NO: 10066, or polynucleotide sequences comprising fragments of SEQ ID NO: 10066.

The invention further comprises an amino acid sequence encoded by SEQ ID NO: 10066, an amino acid sequence having sequence identity to an amino acid sequence encoded by SEQ ID NO: 10066, or an amino acid sequence comprising fragments of an amino acid sequence

encoded by SEQ ID NO: 10066. Preferably, the amino acid sequence comprises SEQ ID NO: 10067.

SEQ ID NO: 9997/9998 demonstrates homology with the a region of `pol1ab` of several coronaviruses. FIGURE 115 shows an alignment of SEQ ID NO^S: 9997/9998 to amino acid sequences for `pol1ab` of bovine coronavirus (SEQ ID NO: 10068), avian infectious bronchitis virus (SEQ ID NO: 10069) and murine hepatitis virus (SEQ ID NO: 10070). A consensus amino acid sequence of SEQ ID NO^S: 9997/9998, SEQ ID NO: 10068, SEQ ID NO: 10069, and SEQ ID NO: 10070 is shown in the bottom row of the alignment in Figure 115 (*e.g.* SEQ ID NO: 10071).

As shown in FIGURE 113, the polynucleotide sequence encoding SEQ ID NO: 9997 has a stop codon after codon 205, between SEQ ID NO^S: 9997 and 9998. Optionally, the stop codon can be removed and the amino acid sequence continued (SEQ ID NO: 10072). Accordingly, the invention provides for an amino acid sequence comprising SEQ ID NO: 9997 and/or SEQ ID NO: 9998, or SEQ ID NO: 10072, and further comprising an amino acid sequence encoding for the C-terminus of a coronavirus `pol1ab` gene or a fragment thereof.

As shown in FIGURE 115, SEQ ID NO^S: 10068, 10069, 10070 and 10071 contain amino acids prior to the N-terminus of SEQ ID NO: 9997. The invention also provides for an amino acid sequence comprising SEQ ID NO: 9997 and further comprising an amino acid sequence encoding for the N-terminus of a coronavirus `pol1ab` protein or a fragment thereof.

The `pol1ab` sequences on FIGURE 115 contain a coding region indicated on the schematic of FIGURE 117 by a "*". In FIGURE 115, the beginning of this genomic region is designated by the arrow crossing in front of amino acid 6080 of the consensus sequence SEQ ID NO: 10071. The end of this genomic region is designated by the arrow crossing in front of amino acid 6604 of the consensus sequence. The invention provides for an amino acid sequence comprising SEQ ID NO: 9997 and/or SEQ ID NO: 9998, or SEQ ID NO: 10072, and further comprising a first amino sequence prior to the N-terminus of said SEQ ID NO: 9997 and/or SEQ ID NO: 9998, or SEQ ID NO: 10072, wherein said first amino acid sequence has homology to an N-terminus sequence of a known coronavirus `pol1ab` "*" protein or a fragment thereof.

The invention further provides for an amino acid sequence comprising SEQ ID NO: 9997 and SEQ ID NO: 9998, wherein the stop codon after SEQ ID NO: 9971 is removed (*i.e.* SEQ ID NO: 10072), and further comprising a second amino acid sequence following the C terminus of SEQ ID NO: 9998, wherein said second amino acid sequence is homologous with a C terminus of a known coronavirus `pol1ab` "*" protein or a fragment thereof.

Examples of such proteins are shown aligned in FIGURE 118, and are SEQ ID NO^S: 10073 to 10077. SEQ ID NO: 10073 comprises SEQ ID NO: 9997 and further comprises amino

acids prior to the N-terminus and subsequent to the C-terminus from the `pol1ab` "*" protein of avian infectious bronchitis virus. SEQ ID NO: 10074 comprises SEQ ID NO: 9997 and further comprises amino acids prior to the N-terminus and subsequent to the C-terminus from the `pol1ab` "*" protein of bovine coronavirus. SEQ ID NO: 10075 comprises SEQ ID NO: 9997 and further comprises amino acids prior to the N-terminus and subsequent to the C-terminus from the `pol1ab` "*" protein of murine hepatitis virus. SEQ ID NO: 10076 comprises SEQ ID NO: 9997 and further comprises amino acids prior to the N-terminus and subsequent to the C-terminus from the consensus of the `pol1ab` "*" protein of avian infectious bronchitis virus, bovine coronavirus, and murine hepatitis virus (FIGURE 115). SEQ ID NO: 10077 comprises the consensus sequence of SEQ ID NOS: 10073 to 10076.

The invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077. The invention further includes an amino acid sequence comprising fragments of an amino acid sequence selected from the group consisting of SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077. The invention further comprises an amino acid sequence with sequence identity to a sequence selected from the group consisting of SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077.

The invention comprises polynucleotides encoding for the amino acid sequences selected from the group consisting of SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077. The invention comprises polynucleotides having sequence identity to polynucleotides encoding for the amino acid sequences selected from the group consisting of SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077. The invention comprises fragments of polynucleotides encoding SEQ ID NO^S: 10073, 10074, 10075, 10076 and 10077.

As shown in Figure 113, SEQ ID NO: 9968 includes a sequence that encodes SEQ ID NO: 10020 followed by a stop codon, giving a C-terminus threonine (Thr) residue. The corresponding sequence from an amino acid sequence encoded by BNI-1 is SEQ ID NO: 10078, which continues past the C-terminus of SEQ ID NO: 10020. Accordingly, the invention includes a protein comprising amino acid sequence SEQ ID NO: 10020 or an amino acid sequence having sequence identity to SEQ ID NO: 10020 or an amino acid sequence comprising a fragment of SEQ ID NO: 10020, wherein the C-terminus residue of said protein is a threonine. Preferably, the C-terminus of said protein is –`ST`. Still more preferably, the C-terminus of said protein is –`EST`. The invention also includes a protein comprising amino acid sequence SEQ ID NO: 10078 or an amino acid sequence having sequence identity to SEQ ID NO: 10078 or an amino acid sequence comprising a fragment of SEQ ID NO: 10078, wherein the C-terminus residue of said protein is Thr. Preferably, the C-terminus of said protein is –`ST`. Still more preferably, the C-terminus of said protein is –`EST`.

SEQ ID NO: 9968 also encodes a 54mer amino acid sequence SEQ ID NO: 10015. The polynucleotide encoding SEQ ID NO: 10015 encodes two stop codons at its C-terminus (Figure 113). The corresponding region from the BNI-1 sequence does not contain this 54mer. Accordingly, the invention includes a protein comprising amino acid sequence SEQ ID NO: 10015, or an amino acid sequence having sequence identity to SEQ ID NO: 10015 or an amino acid sequence comprising a fragment of SEQ ID NO: 10015. The invention further includes a polypeptide comprising SEQ ID NO: 10015 and further comprising a first amino acid sequence prior to the N-terminus of SEQ ID NO: 10015.

SEQ ID NO: 9968 encodes the amino acid sequence SEQ ID NO: 9969. The polynucleotide sequence contains a stop codon at the C-terminus of SEQ ID NO: 9969. Accordingly, the invention includes a protein comprising amino acid sequence SEQ ID NO: 9969, or an amino acid sequence having sequence identity to SEQ ID NO: 9969. The invention further includes a polypeptide comprising SEQ ID NO: 9969 and further comprising a first amino acid sequence prior to the N-terminus of SEQ ID NO: 9969. The invention further includes a polypeptide comprising the sequence SEQ ID NO: 10079.

SEQ ID NO: 9968 encodes amino acid sequence QRT (Figure 113), followed by a stop codon. Accordingly, the invention includes a protein comprising amino acid sequence QRT. The invention further includes a polypeptide comprising amino acid sequence QRT and further comprising a first amino acid sequence prior to the N-terminus of the sequence QRT.

SEQ ID NO: 9968 encodes amino acid sequence SEQ ID NO: 10022, followed by a stop codon at its C-terminus. Accordingly, the invention includes a protein comprising amino acid sequence SEQ ID NO: 10022, or an amino acid sequence having sequence identity to SEQ ID NO: 10022. The invention further includes a polypeptide comprising SEQ ID NO: 10022 and further comprising a first amino acid sequence prior to the N-terminus of SEQ ID NO: 10022.

SEQ ID NO: 9968 encodes amino acid sequence SEQ ID NO: 10027. Within the SEQ ID NO: 10027 coding sequence there are at least three start codons, identified with underlining in Figure 119. The open reading frame indicated by the first start codon is SEQ ID NO: 10081. The open reading frame indicated by the second start codon is SEQ ID NO: 10082. The open reading frame indicated by the third start codon is SEQ ID NO: 10083.

The invention provides a novel SARS polynucleotide sequence SEQ ID NO: 10084. All six reading frames of this 1463mer sequence are shown in Figure 120 (see also Figure 122). The constituent amino acid sequences from Figure 120, having at least 4 amino acids, are listed as SEQ ID NOS: 10085 to 10209 (see Figures 120A to 120F).

The invention includes a polynucleotide sequence comprising SEQ ID NO: 10084. The invention also provides polynucleotide sequences having sequence identity to SEQ ID NO: 10084. The invention also provides for polynucleotide sequences comprising fragments of SEQ

ID NO: 10084. In one embodiment, the polynucleotide fragment does not consist entirely of SEQ ID NO: 10033 or a known coronavirus polynucleotide sequence or a known SARS polynucleotide sequence.

The invention includes an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO: 10084, including the amino acid sequences of Figures 120A to 120F *e.g.* selected from the group consisting of SEQ ID NO^S: 10085 to 10209. Preferably, the amino acid sequence comprises SEQ ID NO: 10149.

The invention also provides amino acid sequences having sequence identity to an amino acid sequence encoded by SEQ ID NO: 10084. The invention provides amino acids having sequence identity to an amino acid sequence from Figures 120A to 120F *e.g.* selected from the group consisting of SEQ ID NO^S: 10085 to 10209.

The invention also provides fragments of amino acid sequences encoded by SEQ ID NO: 10084. The invention also provides fragments of amino acid sequences selected from the group consisting of SEQ ID NO^S: 10085 to 10209. In one embodiment, the fragment does not consist entirely of an amino acid sequence encoded by SEQ ID NO: 10033 or an amino acid sequence of a known coronavirus or an amino acid sequence of a known SARS virus. An alignment of the matching portion of SEQ ID NO: 10033 and SEQ ID NO: 10084 is included in FIGURE 121.

In one embodiment, the invention comprises an amino acid sequence comprising SEQ ID NO: 10149. An alignment of the polynucleotide sequence SEQ ID NO: 10084 to the encoded SEQ ID NO: 10149 is shown in FIGURE 122 (5'3' Frame 3). Analysis of the 5'3' Frame 3 translation by a computer program to predict start codon methionines (NetStart 1.0) (FIGURE 123) reveals SEQ ID NO^S: 10210 to 10215.

The invention includes a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10210, SEQ ID NO: 10211, SEQ ID NO: 10212, SEQ ID NO: 10213, SEQ ID NO: 10214 and SEQ ID NO: 10215. The invention includes a protein having sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10210, SEQ ID NO: 10211, SEQ ID NO: 10212, SEQ ID NO: 10213, SEQ ID NO: 10214 and SEQ ID NO: 10215. In one embodiment, the protein does not consist entirely of an amino acid sequence of a known SARS virus or of a known coronavirus.

The invention includes a fragment of a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10210, SEQ ID NO: 10211, SEQ ID NO: 10212, SEQ ID NO: 10213, SEQ ID NO: 10214 and SEQ ID NO: 10215. In one embodiment, the fragment does not consist entirely of an amino acid sequence of a known SARS virus or of a known coronavirus.

In one embodiment, the invention includes a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 10210, SEQ ID NO: 10211 and

SEQ ID NO: 10212. Partial results of a BLAST of SEQ ID NO: 10210 against GenBank is included in FIGURE 124. These results indicate that SEQ ID NOS: 10210, 10211 and 10212 have functional similarities to a Coronavirus RNA polymerase, particularly the RNA polymerase of murine hepatitis virus, bovine coronavirus, and avian infectious bronchitis.

In one embodiment, the invention is directed to a polypeptide comprising a first amino acid sequence selected from the group consisting of SEQ ID NO: 10210, SEQ ID NO: 10211 and SEQ ID NO: 10212 and a second amino acid sequence from the C-terminus of a coronavirus ORF1ab sequence. Preferably, the second amino acid sequence is from a bovine coronavirus. One example of this embodiment is shown below as SEQ ID NO: 10216. Amino acids 1-481 of SEQ ID NO: 10216 are the first amino acid sequence of SEQ ID NO: 10210, and amino acids 482-1152 are the second amino acid sequence of the C-terminus of a bovine coronavirus orf1ab polyprotein (Gi 26008080) (NP_150073.2) (SEQ ID NO: 10217).

Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 10216. The invention further includes a polypeptide comprising a first amino acid sequence of SEQ ID NO: 10210 and a second amino acid sequence of SEQ ID NO: 10217. The invention further includes a polypeptide comprising a first amino acid sequence having greater than *x*% identity to SEQ ID NO: 10210 and a second amino acid sequence having greater than *y*% identity to SEQ ID NO: 10217, wherein *x* is greater than or equal to 85% (*e.g.*, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) and wherein *y* is greater than or equal to 60% (*e.g.*, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more).

The invention also includes a polypeptide comprising a fragment of SEQ ID NO: 10210, wherein said fragment includes an epitope. Computer-predicted epitopes of SEQ ID NO: 10210, using a 17mer window, are included in FIGURE 125A (Hopp & Woods) and FIGURE 125B (Kyte & Doolittle).

The amino acid sequence of SEQ ID NO: 10210 also contains two predicted glycosylation sites at amino acids 81–84 (NNTE; SEQ ID NO: 10218) and at 180–183 (NHSV; SEQ ID NO: 10219). Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 10210, wherein said fragment includes a glycosylation site. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 10210, wherein said fragment includes the Asn at position 81. Preferably, said Asn is glycosylated. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 10210, wherein said fragment includes the Asn at position 180. Preferably, said Asn is glycosylated.

In one embodiment, the invention includes a polypeptide comprising an amino acid sequence from within Figure 120D and/or SEQ ID NO^S: 10150 to 10160 *e.g.* from SEQ ID NO^S: 10154, 10155, 10158 and 10160. Within SEQ ID NO: 10154 the following amino acid sequences starting with a Met and ending at a stop codon can be identified: SEQ ID NO^S: 10220 to 10227.

Accordingly, the invention includes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10220, SEQ ID NO: 10221, SEQ ID NO: 10222, SEQ ID NO: 10223, SEQ ID NO: 10224, SEQ ID NO: 10225, SEQ ID NO: 10226 and SEQ ID NO: 10227, or a fragment thereof or an amino acid sequence having sequence identity thereto.

In one embodiment, the invention includes a polypeptide comprising the amino acid sequence within Figure 120E *e.g.* from SEQ ID NO^S: 10161 to 10182, and in particular SEQ ID NOS: 10171 and 10176. Within SEQ ID NO^S: 10171 and 10176 the following amino acid sequences starting with a Met and ending at a stop codon can be identified: SEQ ID NO: 10228 and SEQ ID NO: 10229.

Accordingly, the invention includes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10228 and SEQ ID NO: 10229, or a fragment thereof or an amino acid sequence having sequence identity thereto.

In one embodiment, the invention includes a polypeptide comprising an amino acid sequence from Figure 120F *e.g.* SEQ ID NO^S: 10183 to 10209. Within Figure 120F the following amino acid sequence starting with a Met and ending at a stop codon can be identified: SEQ ID NO: 10187. Accordingly, the invention includes a polypeptide comprising an amino acid sequence of SEQ ID NO: 10187, or a fragment thereof or an amino acid sequence having sequence identity thereto.

In one embodiment, the polynucleotides of the invention do not include one of the following primers, disclosed at *http://content.nejm.org/cgi/reprint/NEJMoa030781v2.pdf*:

```
5'GGGTTGGGACTATCCTAAGTGTGA3'  (SEQ ID NO: 10230)
5'TAACACACAACICCATCATCA3'     (SEQ ID NO: 10231)
5'CTAACATGCTTAGGATAATGG3'     (SEQ ID NO: 10232)
5'GCCTCTCTTGTTCTTGCTCGC3'     (SEQ ID NO: 10233)
5'CAGGTAAGCGTAAAACTCATC3'     (SEQ ID NO: 10234)
```

The invention also includes polynucleotide sequences which can be used as probes for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes the polynucleotide primers identified in Table 31 (SEQ ID NO^S: 10235 to 10258), the forward primers SEQ ID NO^S: 10259 to 10281 and the reverse primers SEQ ID NO^S: 10282 to 10298. The invention further includes polynucleotide sequences which are complementary to any one of these primer sequences disclosed herein.

The invention provides a SARS polynucleotide sequence SEQ ID NO: 10299. All six reading frames of this sequence are included in FIGURE 126 (See also Figure 131). The constituent amino acid sequences from Figure 126, having at least 4 amino acids, are listed as SEQ ID NOS: 10300 to 10337.

Accordingly, the invention includes a polynucleotide sequence comprising SEQ ID NO: 10299. It also provides polynucleotide sequences having sequence identity to SEQ ID NO: 10299. The invention also provides for polynucleotide sequences comprising fragments of SEQ ID NO: 10299. In one embodiment, the polynucleotide fragment does not consist entirely of a known polynucleotide sequence of a SARS virus or a known polynucleotide sequence of a coronavirus.

The invention includes an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO: 10299, including the amino acid sequences shown in Figure 126, and the amino acid sequences selected from the group consisting of SEQ ID NO^{S}: 10300 to 10337. Preferably, the amino acid sequence comprises SEQ ID NO: 10316.

The invention also provides amino acid sequences having sequence identity to an amino acid sequence encoded by SEQ ID NO: 10299. The invention provides amino acid sequences having identity to an amino acid sequence selected from the group consisting of SEQ ID NO^{S}: 10300 to 10337.

The invention also provides fragments of amino acid sequences encoded by SEQ ID NO: 10299. The invention also provides fragments of amino acid sequences selected from the group consisting of SEQ ID NO^{S}: 10300 to 10337. In one embodiment, the fragment does not consist entirely of a known amino acid sequence of a SARS virus or a known amino acid sequence of a coronavirus.

In one embodiment, the invention comprises an amino acid sequence comprising SEQ ID NO: 10316. Encoded open reading frames within SEQ ID NO: 10316 include SEQ ID NO: 10338 and SEQ ID NO: 10339.

In one embodiment, the invention comprises an amino acid sequence comprising a sequence from within the 5'3' Frame 1 translation of SEQ ID NO: 10299. The following encoded open reading frame is found within this translation: SEQ ID NO: 10340.

In one embodiment, the invention comprises an amino acid sequence comprising a sequence from within the 3'5' Frame 1 translation of SEQ ID NO: 10299. An encoded open reading frame within this translation is SEQ ID NO: 10341.

In one embodiment, the invention comprises an amino acid sequence comprising a sequence from within the 3'5' Frame 2 translation of SEQ ID NO: 10299. An encoded open reading frame within this translation is SEQ ID NO: 10342.

The invention includes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10338, SEQ ID NO: 10339, SEQ ID NO: 10340, SEQ ID NO: 10341 and SEQ ID NO: 10342. The invention includes a polypeptide having sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10338, SEQ ID NO: 10339, SEQ ID NO: 10340, SEQ ID NO: 10341 and SEQ ID NO: 10342. The invention

includes a fragment of a polypeptide comprising an amino acid sequence elected from the group consisting of SEQ ID NO: 10338, SEQ ID NO: 10339, SEQ ID NO: 10340, SEQ ID NO: 10341 and SEQ ID NO: 10342. In one embodiment, the fragment does not consist entirely of a known SARS virus amino acid sequence or of a known coronavirus amino acid sequence.

In one embodiment, SEQ ID NOS: 10338-10342 are used in fusion proteins. Accordingly, the start codon methionines may be removed. The invention comprises a amino acid sequence selected from the group consisting of SEQ ID NO: 10343, SEQ ID NO: 10344, SEQ ID NO: 10345, SEQ ID NO: 10346 and SEQ ID NO: 10347.

In one embodiment, the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10338 and SEQ ID NO: 10339. Partial BLAST results of SEQ ID NO: 10338 against GenBank are given below:

```
>gi|133593|sp|P18457|RRPB_CVPFS   RNA-DIRECTED RNA POLYMERASE (ORF1B)
 gi|93934|pir||A43489   RNA-directed RNA polymerase (EC 2.7.7.48) - porcine
transmissible gastroenteritis virus (fragment)
 gi|833161|emb|CAA37284.1|      polymerase  [Transmissible  gastroenteritis
virus]
           Length = 533

 Score =  131 bits (329), Expect = 3e-30
 Identities = 55/89 (61%), Positives = 69/89 (77%), Gaps = 1/89 (1%)

Query: 1    MLWCKDGHVETFYPKLQASQAWQPGVAMPNLYKMQRMLLEKCDLQNYGENAVIPKGIMMN 60
            MLWC++ H++TFYP+LQ+++ W PG +MP LYK+QRM LE+C+L NYG    +P GI  N
Sbjct: 217  MLWCENSHIKTFYPQLQSAE-WNPGYSMPTLYKIQRMCLERCNLYNYGAQVKLPDGITTN 275

Query: 61   VAKYTQLCQYLNTLTLAVPSNMRVIHFGA 89
            V KYTQLCQYLNT TL VP  MRV+H GA
Sbjct: 276  VVKYTQLCQYLNTTTLCVPHKMRVLHLGA 304
```

These results indicate that SEQ ID NO: 10338 has functional similarities to an RNA-directed RNA polymerase of porcine transmissible gastroenteritis virus.

Partial BLAST results of SEQ ID NO: 10339 against GenBank are given below:

```
>gb|AAL57305.1| replicase [bovine coronavirus]
          Length = 7094

 Score =  139 bits (351), Expect = 7e-33
 Identities = 64/108 (59%), Positives = 78/108 (72%)

Query: 1      MSVISKVVKVTIDYAEISFMLWCKDGHVETFYPKLQASQAWQPGVAMPNLYKMQRMLLEK 60
              M+ +SKVV V +D+ +  FMLWC D  V TFYP+LQA+  W+PG +MP LYK     +E+
Sbjct:   6760   LNCVSKVVNVNVDFKDFQFMLWCNDEKVMTFYPRLQAASDWKPGYSMPVLYKYLNSPMER
6819

Query: 61    CDLQNYGENAVIPKGIMMNVAKYTQLCQYLNTLTLAVPSNMRVIHFGA 108
               L NYG+    +P G MMNVAKYTQLCQYLNT TLAVP NMRV+H GA
Sbjct: 6820 VSLWNYGKPVTLPTGCMMNVAKYTQLCQYLNTTTLAVPVNMRVLHLGA 6867
```

These results indicate that SEQ ID NO: 10339 has functional similarities to a replicase of bovine coronavirus.

The SARS virus may contain polymorphism at the Glu-20 residue of SEQ ID NO: 10338. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 10338, wherein said polypeptide includes an amino acid sequence selected from the group consisting of ASQAW (SEQ ID NO: 10348) and ASRAW (SEQ ID NO: 10349). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 10338, wherein said fragment includes an amino acid sequence selected from the group consisting of SEQ ID NO: 10348 and SEQ ID NO: 10349.

The SARS virus may contain polymorphism at the Ser-80 residue of SEQ ID NO: 10338. below. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 10338, wherein said polypeptide includes an amino acid sequence selected from the group consisting of VPSNM (SEQ ID NO: 10350) and VPTNM (SEQ ID NO: 10351). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 10338, wherein said fragment includes an amino acid sequence selected from the group consisting of SEQ ID NO: 10350 and SEQ ID NO: 10351.

The invention also includes polynucleotide sequences which can be used as probes for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in Table 32. The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in Table 32.

The invention provides a SARS polynucleotide sequence SEQ ID NO: 10505. All six reading frames of this sequence are shown in Figure 127 (see also Figure 132). The constituent amino acid sequences from Figure 127, having at least 4 amino acids, are listed as SEQ ID NOS: 10506 to 10570.

The invention includes a polynucleotide sequence comprising SEQ ID NO: 10505. The invention also provides polynucleotide sequences having sequence identity to SEQ ID NO: 10505. The invention also provides for polynucleotide sequences comprising fragments of SEQ ID NO: 10505. In one embodiment, the polynucleotide fragment does not consist entirely of a known SARS virus polynucleotide sequence or of a known coronavirus polynucleotide sequence.

The invention includes an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO: 10505, including the amino acid sequences shown in Figure 127, and particularly those selected from the group consisting of SEQ ID NO^{S}: 10506 to 10570. Preferably, the amino acid sequence comprises SEQ ID NO: 10532 and/or SEQ ID NO: 10533.

The invention also provides amino acid sequences having sequence identity to an amino acid sequence encoded by SEQ ID NO: 10505. The invention provides amino acid sequences

As used herein, the term "target nucleic acid region" or "target nucleic acid" denotes a nucleic acid molecule with a "target sequence" to be amplified. The target nucleic acid may be either single-stranded or double-stranded and may include other sequences besides the target sequence, which may not be amplified. The term "target sequence" refers to the particular nucleotide sequence of the target nucleic acid which is to be amplified. The target sequence may include a probe-hybridizing region contained within the target molecule with which a probe will form a stable hybrid under desired conditions. The "target sequence" may also include the complexing sequences to which the oligonucleotide primers complex and be extended using the target sequence as a template. Where the target nucleic acid is originally single-stranded, the term "target sequence" also refers to the sequence complementary to the "target sequence" as present in the target nucleic acid. If the "target nucleic acid" is originally double-stranded, the term "target sequence" refers to both the plus (+) and minus (–) strands.

The term "primer" or "oligonucleotide primer" as used herein, refers to an oligonucleotide which acts to initiate synthesis of a complementary DNA strand when placed under conditions in which synthesis of a primer extension product is induced *i.e.* in the presence of nucleotides and a polymerization-inducing agent such as a DNA or RNA polymerase and at suitable temperature, pH, metal concentration, and salt concentration. The primer is preferably single-stranded for maximum efficiency in amplification, but may alternatively be double-stranded. If double-stranded, the primer is first treated to separate its strands before being used to prepare extension products. This denaturation step is typically effected by heat, but may alternatively be carried out using alkali, followed by neutralization. Thus, a "primer" is complementary to a template, and complexes by hydrogen bonding or hybridization with the template to give a primer/template complex for initiation of synthesis by a polymerase, which is extended by the addition of covalently bonded bases linked at its 3' end complementary to the template in the process of DNA synthesis.

As used herein, the term "probe" or "oligonucleotide probe" refers to a structure comprised of a polynucleotide, as defined above, that contains a nucleic acid sequence complementary to a nucleic acid sequence present in the target nucleic acid analyte. The polynucleotide regions of probes may be composed of DNA, and/or RNA, and/or synthetic nucleotide analogs. When an "oligonucleotide probe" is to be used in a 5' nuclease assay, such as the TaqMan™ technique, the probe will contain at least one fluorescer and at least one quencher which is digested by the 5' endonuclease activity of a polymerase used in the reaction in order to detect any amplified target oligonucleotide sequences. In this context, the oligonucleotide probe will have a sufficient number of phosphodiester linkages adjacent to its 5' end so that the 5' to 3' nuclease activity employed can efficiently degrade the bound probe to separate the fluorescers and quenchers.

When an oligonucleotide probe is used in the TMA technique, it will be suitably labeled, as described below.

It will be appreciated that the hybridizing sequences need not have perfect complementarity to provide stable hybrids. In many situations, stable hybrids will form where fewer than about 10% of the bases are mismatches, ignoring loops of four or more nucleotides. Accordingly, as used herein the term "complementary" refers to an oligonucleotide that forms a stable duplex with its "complement" under assay conditions, generally where there is about 90% or greater homology.

The terms "hybridize" and "hybridization" refer to the formation of complexes between nucleotide sequences which are sufficiently complementary to form complexes via Watson-Crick base pairing. Where a primer "hybridizes" with target (template), such complexes (or hybrids) are sufficiently stable to serve the priming function required by *e.g.* the DNA polymerase to initiate DNA synthesis.

Stringent hybridization conditions will typically include salt concentrations of less than about 1 M, more usually less than about 500 mM and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, more typically greater than about 30°C, and preferably in excess of about 37°C. Longer fragments may require higher hybridization temperatures for specific hybridization. Other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents and extent of base mismatching, and the combination of parameters used is more important than the absolute measure of any one alone. Other hybridization conditions which may be controlled include buffer type and concentration, solution pH, presence and concentration of blocking reagents to decrease background binding such as repeat sequences or blocking protein solutions, detergent type(s) and concentrations, molecules such as polymers which increase the relative concentration of the polynucleotides, metal ion(s) and their concentration(s), chelator(s) and their concentrations, and other conditions known in the art. Less stringent, and/or more physiological, hybridization conditions are used where a labeled polynucleotide amplification product cycles on and off a substrate linked to a complementary probe polynucleotide during a real-time assay which is monitored during PCR amplification such as a molecular beacon assay. Such less stringent hybridization conditions can also comprise solution conditions effective for other aspects of the method, for example reverse transcription or PCR.

As used herein, a "biological sample" refers to a sample of tissue, cells or fluid isolated from a subject, that commonly includes antibodies produced by the subject. Typical samples include but are not limited to, blood, plasma, serum, fecal matter, urine, bone marrow, bile, spinal fluid, lymph fluid, samples of the skin, secretions of the skin, respiratory, intestinal, and

genitourinary tracts, tears, saliva, sputum, mucous, milk, blood cells, organs, tissues, biopsies (*e.g.* lung, liver, kidney) and also samples of *in vitro* cell culture constituents including but not limited to conditioned media resulting from the growth of cells and tissues in culture medium *e.g.* recombinant cells, and cell components. Other samples that may be used for diagnosis include stool samples and nasopharyngeal aspirates.

The term "antibody" encompasses polyclonal and monoclonal antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, chimeric antibodies and, humanized antibodies, as well as: hybrid (chimeric) antibody molecules (see, for example, Winter *et al.* (1991) *Nature* 349:293-299; and US Patent 4,816,567); F(ab')$_2$ and F(ab) fragments; Fv molecules (noncovalent heterodimers, see, for example, Inbar *et al.* (1972) *Proc Natl Acad Sci USA* 69:2659-2662; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096); single-chain Fv molecules (sFv) (*see, e.g.*, Huston *et al.* (1988) *Proc Natl Acad Sci USA* 85:5879-5883); oligobodies; dimeric and trimeric antibody fragment constructs; minibodies (see, *e.g.*, Pack *et al.* (1992) *Biochem* 31:1579-1584; Cumber *et al.* (1992) *J Immunology* 149B:120-126); humanized antibody molecules (see, *e.g.*, Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyan *et al.* (1988) *Science* 239:1534-1536; and UK Patent Publication No. GB 2,276,169, published 21 September 1994); and, any functional fragments obtained from such molecules, wherein such fragments retain specific-binding properties of the parent antibody molecule.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins.

Methods of making polyclonal and monoclonal antibodies are known in the art. Polyclonal antibodies are generated by immunizing a suitable animal, such as a mouse, rat, rabbit, sheep or goat, with an antigen of interest. In order to enhance immunogenicity, the antigen can be linked to a carrier prior to immunization. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, *etc.*, in order to enhance the immunogenicity thereof.

Rabbits, sheep and goats are preferred for the preparation of polyclonal sera when large volumes of sera are desired. These animals are good design choices also because of the availability of labeled anti-rabbit, anti-sheep and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the antigen in saline, preferably in an adjuvant such as

Freund's complete adjuvant ("FCA"), and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). The animal is generally boosted 2-6 weeks later with one or more injections of the antigen in saline, preferably using Freund's incomplete adjuvant ("FIA"). Antibodies may also be generated by in vitro immunization, using methods known in the art. Polyclonal antisera is then obtained from the immunized animal.

Monoclonal antibodies are generally prepared using the method of Kohler & Milstein (1975) *Nature* 256:495-497, or a modification thereof, as described above.

Nucleic acid detection methods

There are many well known methods of amplifying targeted sequences, such as the polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), the ligase chain reaction (LCR), the strand displacement amplification (SDA), and the nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA) to name a few. These methods are described generally in the following references: (PCR) US Patents 4,683,195, 4,683,202, and 4,800,159; (RT-PCR) US patent 5,310,652, 5,322,770; (LCR) EP Application No., 320,308 published Jun. 14, 1989; (SDA) US Pat. Nos. 5,270,184, and 5,455,166 and "Empirical Aspects of Strand Displacement Amplification" by G. T. Walker in *PCR Methods and Applications*, 3(1):1-6 (1993), Cold Spring Harbor Laboratory Press; (TMA) US Patent No. 5,399,491, and (NASBA) "Nucleic Acid Sequence-Based Amplification (NASBA™)" by L. Malek *et al.*, Ch. 36 in *Methods in Molecular Biology*, Vol. 28: Protocols for Nucleic Acid Analysis by Nonradioactive Probes, 1994 Ed. P. G. Isaac, Humana Press, Inc., Totowa, N.J. PCR methods may include variations that permit quantitation of the target sequence, for example, by real time PCR analysis (e.g., as described in US patents 5,210,015, 5,487,972, 5,994,056, 6,171,785 inter alia). (Each of the above references are hereby incorporated by reference).

One embodiment of the method of the invention for detecting the presence of SARS virus in a sample comprises providing a sample suspected of containing a SARS virus nucleic acid target, amplifying a template sequence contained within said SARS virus nucleic acid target by any known technique of nucleic acid amplification, including any of those mentioned herein, using the oligonucleotide primers described herein, particularly those primers comprising the kits described herein, and detecting the amplified template sequence, wherein the presence of the amplified template sequence indicates the presence of SARS virus in said sample.

Amplification techniques generally involve the use of two primers. Where a target sequence is single-stranded, the techniques generally involve a preliminary step in which a complementary strand is made in order to give a double-stranded target. The two primers hybridize to different strands of the double-stranded target and are then extended. The extended products can serve as targets for further rounds of hybridization/extension. The net effect is to amplify a template sequence within the target, the 5' and 3' termini of the template being defined

by the locations of the two primers in the target. As an alternative, if one or both of the primers contains a promoter sequence then the target can be amplified (by transcription) using a RNA polymerase (as in TMA).

The present invention provides methods and kits for amplifying and/or detecting a template or target sequence in the SARSV viral nucleic acid. The invention provides a kit comprising primers for amplifying a template sequence contained within a SARSV nucleic acid target, the kit comprising a first primer and a second primer, wherein the first primer comprises a sequence substantially complementary to a portion of said template sequence and the second primer comprises a sequence substantially complementary to a portion of the complement of said template sequence, wherein the sequences within said primers which have substantial complementarity define the termini of the template sequence to be amplified.

Kits of the invention may further comprise a probe which is substantially complementary to the template sequence and/or to its complement and which can hybridize thereto. This probe can be used in a hybridization technique to detect amplified template, or to isolate (*i.e.* "capture) the amplified template or the original target nucleic acid.

Kits of the invention may further comprise primers and/or probes for generating and detecting an internal standard, in order to aid quantitative measurements (*e.g* Fille *et al.* 1997 *Biotechniques* 23:34-36).

Kits of the invention may further comprise a DNA polymerase, which will generally be a thermostable DNA polymerase where a non-isothermal amplification process is to be used. The kits may also comprise supplies of dNTPs, a magnesium salt (*e.g.* $MgCl_2$), buffer solutions, *etc.*

Kits of the invention may comprise more than one pair of primers (*e.g.* for nested amplification), and one primer may be common to more than one primer pair. The kit may also comprise more than one probe.

<u>Oligomer Probes and Primers</u>

In connection with the nucleic acid detection methods of the present invention described above, oligomers having sequence similarity, or complementarity, to the SARSV genome are useful. The SARSV genome sequences mentioned herein may be used to produce probes and primers which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of those of ordinary skill in the art. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multi-gene family or in related species like mouse and man.

Using as a basis the SARSV genome which can be found as described herein, and/or preferably conserved regions of the SARSV genome, and/or the particularly described primer and probe sequences as disclosed herein, oligomers of approximately 8 nucleotides or more can be prepared which hybridize with the positive strand(s) of SARSV RNA or its complement, as well as to SARSV cDNAs. These oligomers can serve as probes for the detection (including isolation and/or labeling) of polynucleotides which contain SARSV nucleotide sequences, and/or as primers for the transcription and/or replication of targeted SARSV sequences. The oligomers contain a targeting polynucleotide sequence, which is comprised of nucleotides which are complementary to a target SARSV nucleotide sequence; the sequence is of sufficient length and complementarity with the SARSV sequence to form a duplex which has sufficient stability for the purpose intended. For example, if the purpose is the isolation, via immobilization, of an analyte containing a target SARSV sequence, the oligomers would contain a polynucleotide region which is of sufficient length and complementarity to the targeted SARSV sequence to afford sufficient duplex stability to immobilize the analyte on a solid surface, via its binding to the oligomers, under the isolation conditions. For example, also, if the oligomers are to serve as primers for the transcription and/or replication of target SARSV sequences in an analyte polynucleotide, the oligomers would contain a polynucleotide region of sufficient length and complementarity to the targeted SARSV sequence to allow the polymerizing agent to continue replication from the primers which are in stable duplex form with the target sequence, under the polymerizing conditions. For example, also, if the oligomers are to be used as label probes, or are to bind to multimers, the targeting polynucleotide region would be of sufficient length and complementarity to form stable hybrid duplex structures with the label probes and/or multimers to allow detection of the duplex. The oligomers may contain a minimum of about 4 contiguous nucleotides which are complementary to targeted SARSV sequence; usually the oligomers will contain a minimum of about 8 contiguous nucleotides which are complementary to the targeted SARSV sequence, and preferably will contain a minimum of about 14, 15, 16, 17, 18, 19, 20,21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides and up to about 50, 75, 100, 200 contiguous nucleotides or more, which are complementary to the targeted SARSV sequence.

Typically, for use in the amplification based methods (for example, PCR, RT-PCR, TMA) oligomers will be used as primer sets such that one member of the primer set has sequence similarity or complementarity to a more conserved (among coronaviruses) portion of the SARSV genome and the other member of the primer set has sequence similarity or complementarity to a less conserved portion. The primer sets can be used to amplify the target region in ways that are well known in the art. Typically, the 5' untranslated region (5'UTR) and the 3' untranslated region (3'UTR) are among the most conserved regions. Figure 8 shows an alignment of the 5'UTR of several coronaviruses. Figure 10 shows an alignment of the 3'UTR of several

coronaviruses. Figures 9 and 11 show the sequences of preferred primers for amplification of the 5'UTR and 3'UTR, respectively. Other primers and probes can readily be designed based on the sequence alignments provided herein.

The oligomer, however, need not consist only of the sequence which is complementary to the targeted SARSV sequence. It may contain in addition, nucleotide sequences (*e.g.*promoters) or other moieties which are suitable for the purposes for which the oligomers are used. For example, if the oligomers are used as primers for the amplification of SARSV sequences via, for example, PCR, they may contain sequences which, when in duplex, form restriction enzyme sites which facilitate the cloning of the amplified sequences. For example, also, if the oligomers are to be used as "capture probes" in hybridization assays, they would contain in addition a binding partner which is coupled to the oligomer containing the nucleotide sequence which is complementary to the targeted SARSV sequence. Other types of moieities or sequences which are useful of which the oligomers may be comprised or coupled to, are those which are known in the art to be suitable for a variety of purposes, including the labeling of nucleotide probes.

Table 4 (SEQ ID NOS: 1021-6020) shows forward and reverse primers that are useful for nucleic acid amplification of SARSV for diagnostic and screening methods.

Preferred primers and probes for SARS nucleic acid detection for diagnostic and screening are SEQ ID NOS: 7332-7336 (forward primers), SEQ ID NOS: 7337-7341 (reverse primers) and SEQ ID NOS: 7342-7352 (probes). These primers and probes are useful for detection of sequences in the 3' UTR.

Any of the above forward primers may be used in combination with any of the above reverse primers for amplification of SARSV nucleic acid. The amplified product may be detected (or captured) with any of the above probes. Particularly preferred combinations of forward and reverse primers and the probes for detecting the amplified product include: Forward SEQ ID NO: 7332 with reverse SEQ ID NO: 7337, 7338, 7339 or 7341 and probe SEQ ID NO: 7342; forward SEQ ID NO: 7333 or 7334 with reverse SEQ ID NO: 7340 and any of probes SEQ ID NO: 7343-7351; Forward SEQ ID NO: 7335 and reverse SEQ ID NO: 7340 or 7341 and any of probes SEQ ID NO: 7342-7352. Other combinations of forward and reverse primers and appropriate probes can readily be determined by those skilled in the art from the above information.

Additional preferred primers and probes for SARS nucleic acid detection for diagnostic and screening are SEQ ID NOS: 7353-7362 (forward primers), SEQ ID NOS: 7363-7373 (reverse primers) and SEQ ID NOS: 7374-7385 (probes). The primers and probes are useful for detection of sequences in the 5' UTR.

The above primers may be used in combination for amplification of SARSV nucleic acid as follows: any of forward primers SEQ ID NO: 7353-7356 with any of reverse primers SEQ ID

NO: 7363-7366, 7368 and the amplified product detected (or captured) with probes SEQ ID NO: 7374; any of forward primers SEQ ID NO: 7357-7362 with any of reverse primers SEQ ID NO: 7367, 7369-7373 and the amplified products detected (or captured) with any of probes SEQ ID NO: 7375-7385. Particularly preferred combinations of forward and reverse primers and probes are: Forward primers SEQ ID NO: 7353-7356 with any of reverse primers SEQ ID NO: 7363-7366 and probes SEQ ID NO: 7374; forward primers SEQ ID NO: 7357-7358 with reverse primers SEQ ID NO: 7367, 7369 and probes SEQ ID NO: 7375 or 7376; Forward primers SEQ ID NO: 7357-7359 with reverse primers SEQ ID NO: 7367, 7369 or 7370 and probe SEQ ID NO: 7375 or 7376. More preferred are combinations of SEQ ID NO: 7353 or 7354 with SEQ ID NO: 7363 or 7364 and probe SEQ ID NO: 7374. Other combinations of forward and reverse primers and appropriate probes can readily be determined by those skilled in the art from the above information. A particularly conserved octanucleotide sequence (SEQ ID NO: 7386) occurs in the 3'UTR of SARS (approximately 70-80 bases from the 3' end) and of several other Coronaviruses that may be particularly useful in identifying SARSV. Primers including in this region are preferably combined with reverse primers from regions of sequence that are more specific for SARS.

In addition to the above, the intergenic sequence (IS) that is characteristic of Coronavirus has been identified in SARSV (see above). The IS minimally comprises the sequence ACGAAC (SEQ ID NO: 7293) which occurs upstream of each open reading frame (ORF) in the viral genome. The 5'UTR which includes the IS is spliced onto the 5' end of each viral mRNA at or adjacent to the site of the IS. Thus, primers comprising the IS or its complement are useful for amplifying viral nucleic acids, including cDNA made from the viral RNAs. The invention thus comprises a set of primers in which one primer comprises ACGAAC (SEQ ID NO: 7293) or its complement (SEQ ID NO: 7387) and one primer comprises any appropriate sequence from the SARS genome, or a complementary sequence. Useful probes for detecting and/or capturing the viral RNAs or cDNA made from the viral RNAs may also comprise the IS sequence, or its complement, described above.

One set of primers for amplification of SARS sequences, particularly by RT-PCR, uses SEQ ID NOs 6562, 6563, 6564 and 6565. Of these, 6562 & 6564 are sense primers and 6563 & 6565 are antisense primers. Primers SEQ ID NOS: 6562 & 6565 may be used in a first amplification, with a second nested amplification being performed using primers SEQ ID NOS: 6563 & 6564. In some embodiments of the invention, these four primers are excluded.

One kit for amplification and detection of SARS sequences, particularly by RT-PCR, uses SEQ ID NOs 6567 & 6568 as primers, and SEQ ID NO 6566 as a probe (typically labeled *e.g.* with TAMRA and/or FAM) for the amplified sequence. In some embodiments of the invention, these primers and probe are excluded.

One kit for amplification and detection of SARS sequences, particularly by RT-PCR, uses SEQ ID NOs 7395 & 6568 as primers, and SEQ ID NO 6566 as a probe (typically labeled *e.g.* with TAMRA and/or FAM) for the amplified sequence. In some embodiments of the invention, these primers and probe are excluded.

One kit for amplification of SARS sequences, particularly the nucleocapsid gene, uses SEQ ID NOs 6560 & 6561 as primers. In some embodiments of the invention, these primers are excluded.

One kit for amplification of SARS sequences uses SEQ ID NOs 6496, 6497, 6562, 6563, 6564 & 6565 as primers. In some embodiments of the invention, these primers are excluded.

One kit for amplification of SARS sequences uses SEQ ID NOs 6562, 6563, 6564 & 6565 as primers. In some embodiments of the invention, these primers are excluded.

One kit for amplification of SARS sequences uses SEQ ID NOs 6500, 6501, 6502 & 6503 as primers. In some embodiments of the invention, these primers are excluded.

One kit for amplification of SARS sequences uses SEQ ID NOs 6496, 6497, 6500, 6501, 6502, 6503, 6562, 6563, 6564 & 6565 as primers. In some embodiments of the invention, these primers are excluded.

One kit for amplification and detection of SARS sequences, particularly by realtime (*e.g.* TaqMan™) PCR, uses SEQ ID NOs 6567 & 6568 as primers, and SEQ ID NO 6566 as a probe (typically labeled *e.g.* with TAMRA and/or FAM) for the amplified sequence. In some embodiments of the invention, these primers and probe are excluded.

One kit for amplification and detection of SARS sequences, particularly by realtime (*e.g.* TaqMan™) PCR, uses SEQ ID NOs 7395 & 6568 as primers, and SEQ ID NO 6566 as a probe (typically labeled *e.g.* with TAMRA and/or FAM) for the amplified sequence. In some embodiments of the invention, these primers and probe are excluded.

One kit for amplification and detection of SARS sequences uses SEQ ID NOs 6562, 6565 and 6568 as primers, and SEQ ID NOs 7396 and 7397 as probes (typically labeled *e.g.* with TAMRA and/or FAM) for the amplified sequence. In some embodiments of the invention, these primers and probe are excluded.

One kit for amplification and detection of SARS sequences uses an oligonucleotide comprising SEQ ID NO: 9780 as a forward primer, an oligonucleotide comprising SEQ ID NO: 9781 as a reverse primer, and an oligonucleotide comprising SEQ ID NO: 9782 as a probe.

Preferred sequences for use with RT-PCR and LightCycler analysis include SEQ ID NOs 6562, 6568, 6565, 7396 & 7397. In some embodiments of the invention, these primers and probe are excluded.

The preparation of the oligomers is by means known in the art, including, for example, by methods which include excision, transcription, or chemical synthesis. The target sequences

and/or regions of the genome which are selected to which the targeting polynucleotides of the oligomers are complementary depend upon the purpose. For example, if the goal is to screen for the presence of SARSV in biological samples (*e.g.* blood, respiratory material, liver, lung), the preferred oligomers would be used as probes and/or primers, and would hybridize to conserved regions of the SARSV genome. Some of the conserved regions of the SARSV genome to which the oligomers may bind are described herein, for example, 5'UTR and 3'UTR.

In the basic nucleic acid hybridization assay, single-stranded analyte nucleic acid (either DNA or RNA) is hybridized to a nucleic acid probe, and resulting duplexes are detected. The probes for SARSV polynucleotides (natural or derived) are a length which allows the detection of unique viral sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and about 13, 14, 15, 16, 17, 18, 19, 20, or 21 or more nucleotides or more appears optimal. Preferably, these sequences will derive from regions which lack heterogeneity. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. Among useful probes, for example, are those derived from less conserved regions of the SARSV genome. Regions of the genome that are typically less conserved can be readily ascertained from the sequence alignments provided herein, as well as by any other well known techniques. A complement to any unique portion of the SARSV genome will be satisfactory. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased.

For use of such probes as agents to detect the presence of SARSV polynucleotides (for example in screening for contaminated blood or for diagnosing infected individuals), the biological sample to be analyzed, such as, without limitation, blood, serum, lung, liver, mucous, kidney, saliva, or sputum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. In order to form hybrid duplexes with the targeting sequence of the probe, the targeted region of the analyte nucleic acid must be in single stranded form. Where the sequence is naturally present in single stranded form, denaturation will not be required. However, where the sequence is present in double stranded form, the sequence will be denatured. Denaturation can be carried out by various techniques known in the art. Subsequent to denaturation, the analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte, and the resulting duplexes containing the probe(s) are detected.

Detection of the resulting duplex, if any, is usually accomplished by the use of labeled probes; alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling

probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (*e.g.*, nick translation or kinasing), biotin, fluorescent groups, chemiluminescent groups (*e.g.*, dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, and the like.

The region of the probes which are used to bind to the analyte can be made completely complementary to the SARSV genome. Therefore, usually high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency should only be used if the probes are complementary to regions of the viral genome which lack heterogeneity. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis T. (1982).

Variations of this basic scheme which are known in the art, including those which facilitate separation of the duplexes to be detected from extraneous materials and/or which amplify the signal from the labeled moiety, may also be used. A number of these variations are reviewed in, for example: Matthews & Kricka (1988), *Analytical Biochemistry* 169:1; Landegren *et al.* (1988), Science 242:229; and Mittlin (1989), *Clinical Chem.* 35:1819. These and the following publications describing assay formats are hereby incorporated by reference herein. Probes suitable for detecting SARSV in these assays are comprised of sequences which hybridize with target SARSV polynucleotide sequences to form duplexes with the analyte strand, wherein the duplexes are of sufficient stability for detection in the specified assay system.

A suitable variation is, for example, one which is described in US Pat. No. 4,868,105, issued Sep. 9, 1989, and in EPO Publication No. 225,807 (published Jun. 16, 1987). These publications describe a solution phase nucleic acid hybridization assay in which the analyte nucleic acid is hybridized to a labeling probe set and to a capturing probe set. The probe-analyte complex is coupled by hybridization with a solid-supported capture probe that is complementary to the capture probe set. This permits the analyte nucleic acid to be removed from solution as a solid phase complex. Having the analyte in the form of a solid phase complex facilitates subsequent separation steps in the assay. The labeling probe set is complementary to a labeled probe that is bound through hybridization to the solid phase/analyte complex.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is

repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in US Pat. Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target strand, and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3' hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more completely in EP-A-320 308 to K. Backman published Jun. 16, 1989 and EP-A-0439182 to K. Backman *et al.*, published Jul. 31, 1991, both of which are incorporated herein by reference.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in US Pat. No. 5,322,770, which is incorporated herein by reference; or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall *et al.*, PCR Methods and Applications 4:80-84 (1994), which also is incorporated herein by reference.

TMA is described in detail in, *e.g.*, US Patent No. 5,399,491, the disclosure of which is incorporated herein by reference in its entirety. In one example of a typical assay, an isolated nucleic acid sample, suspected of containing a SARSV target sequence, is mixed with a buffer concentrate containing the buffer, salts, magnesium, nucleotide triphosphates, primers, dithiothreitol, and spermidine. The reaction is optionally incubated at about 100°C for approximately two minutes to denature any secondary structure. After cooling to room temperature, reverse transcriptase, RNA polymerase, and RNAse H are added and the mixture is incubated for two to four hours at 37°C. The reaction can then be assayed by denaturing the product, adding a probe solution, incubating 20 minutes at 60°C, adding a solution to selectively hydrolyze the unhybridized probe, incubating the reaction six minutes at 60°C, and measuring the remaining chemiluminescence in a luminometer.

Generally, TMA includes the following steps: (a) isolating nucleic acid, including RNA, from the biological sample of interest suspected of being infected with SARSV; and (b) combining into a reaction mixture (i) the isolated nucleic acid, (ii) first and second oligonucleotide primers, the first primer having a complexing sequence sufficiently complementary to the 3' terminal portion of an RNA target sequence, if present (for example the (+) strand), to complex therewith, and the second primer having a complexing sequence sufficiently complementary to the 3' terminal portion of the target sequence of its complement (for example, the (-) strand) to complex therewith, wherein the first oligonucleotide further comprises a sequence 5' to the complexing sequence which includes a promoter, (iii) a reverse transcriptase or RNA and DNA dependent DNA polymerases, (iv) an enzyme activity which selectively degrades the RNA strand of an RNA-DNA complex (such as an RNAse H) and (v) an RNA polymerase which recognizes the promoter.

The components of the reaction mixture may be combined stepwise or at once. The reaction mixture is incubated under conditions whereby an oligonucleotide/target sequence is formed, including DNA priming and nucleic acid synthesizing conditions (including ribonucleotide triphosphates and deoxyribonucleotide triphosphates) for a period of time sufficient to provide multiple copies of the target sequence. The reaction advantageously takes place under conditions suitable for maintaining the stability of reaction components such as the component enzymes and without requiring modification or manipulation of reaction conditions during the course of the amplification reaction. Accordingly, the reaction may take place under conditions that are substantially isothermal and include substantially constant ionic strength and pH. The reaction conveniently does not require a denaturation step to separate the RNA-DNA complex produced by the first DNA extension reaction.

Suitable DNA polymerases include reverse transcriptases, such as avian myeloblastosis virus (AMV) reverse transcriptase (available from, *e.g.*, Seikagaku America, Inc.) and Moloney murine leukemia virus (MMLV) reverse transcriptase (available from, *e.g.*, Bethesda Research Laboratories).

Promoters or promoter sequences suitable for incorporation in the primers are nucleic acid sequences (either naturally occurring, produced synthetically or a product of a restriction digest) that are specifically recognized by an RNA polymerase that recognizes and binds to that sequence and initiates the process of transcription whereby RNA transcripts are produced. The sequence may optionally include nucleotide bases extending beyond the actual recognition site for the RNA polymerase which may impart added stability or susceptibility to degradation processes or increased transcription efficiency. Examples of useful promoters include those which are recognized by certain bacteriophage polymerases such as those from bacteriophage

T3, T7 or SP6, or a promoter from *E. coli*. These RNA polymerases are readily available from commercial sources, such as New England Biolabs and Epicentre.

Some of the reverse transcriptases suitable for use in the methods herein have an RNAse H activity, such as AMV reverse transcriptase. It may, however, be preferable to add exogenous RNAse H, such as *E. coli* RNAse H, even when AMV reverse transcriptase is used. RNAse H is readily available from, *e.g.*, Bethesda Research Laboratories.

The RNA transcripts produced by these methods may serve as templates to produce additional copies of the target sequence through the above-described mechanisms. The system is autocatalytic and amplification occurs autocatalytically without the need for repeatedly modifying or changing reaction conditions such as temperature, pH, ionic strength or the like.

Detection may be done using a wide variety of methods, including direct sequencing, hybridization with sequence-specific oligomers, gel electrophoresis and mass spectrometry. these methods can use heterogeneous or homogeneous formats, isotopic or nonisotopic labels, as well as no labels at all.

Suitable labeling moieties for attachment to primers and/or to probes used in methods of the invention include, but are not limited to: 5-FAM (also called 5-carboxyfluorescein; also called Spiro(isobenzofuran-1(3H), 9'-(9H)xanthene)-5-carboxylic acid,3',6'-dihydroxy-3-oxo-6-carboxyfluorescein); 5-Hexachloro-Fluorescein ([4,7,2',4',5',7'-hexachloro-(3',6'-dipivaloylfluoresceinyl)-6-carboxylic acid]); 6-Hexachloro-Fluorescein ([4,7,2',4',5',7'-hexachloro-(3',6'-dipivaloylfluoresceinyl)-5-carboxylic acid]); 5-Tetrachloro-Fluorescein ([4,7,2',7'-tetrachloro-(3',6'-dipivaloylfluoresceinyl)-5- carboxylic acid]); 6-Tetrachloro-Fluorescein ([4,7,2',7'-tetrachloro-(3',6'-dipivaloylfluoresceinyl)-6- carboxylic acid]); tetramethylrhodamines (TAMRA), including (i) 5-TAMRA (5-carboxytetramethylrhodamine; Xanthylium, 9-(2,4-dicarboxyphenyl)-3,6- bis(dimethylamino) and (ii) 6-TAMRA (6-carboxytetramethylrhodamine; Xanthylium, 9-(2,5-dicarboxyphenyl)-3,6- bis(dimethylamino); EDANS (5-((2-aminoethyl)amino)naphthalene- 1-sulfonic acid) ; 1,5-IAEDANS (5-((((2-iodoacetyl)amino)ethyl) amino)naphthalene-1-sulfonic acid); DABCYL (4-((4-(dimethylamino)phenyl) azo)benzoic acid); Cy5 (Indodicarbocyanine-5); Cy3 (Indodicarbocyanine-3); and BODIPY™ FL (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid). Labelling of probes with both FAM (*e.g.* at 5') and TAMRA (*e.g.* at 3') is preferred.

Nucleic acids of the invention may be used in solution or may be bound to a solid matrix or support *e.g.* in the format of a DNA array,

As is readily apparent, design of the assays described herein are subject to a great deal of variation, and many formats are known in the art. The above descriptions are merely provided as

guidance and one of skill in the art can readily modify the described protocols, using techniques well known in the art.

One 302nt amplicon of the SARS virus is known as "BNI-1" (SEQ ID NO: 9927). It was sequenced at the Bernhard Nocht Institute, Hamburg, Germany. In April 2003 the BNI-1 sequence was published on the WHO website (*http://www.who.int/csr/sars/primers/en/*) and in Dorsten *et al.*, "Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome", *New England Journal of Medicine*, published online at *http://www.nejm.org*. Both references are incorporated herein by reference in their entirety. Some embodiments of the invention do not encompass a nucleic acid consisting of SEQ ID NO: 9927. Some other embodiments of the invention do not encompass a nucleic acid comprising SEQ ID NO: 9927. Some embodiments of the invention do not encompass a polypeptide consisting of any one of SEQ ID NO^{S}: 9928 to 9959. Some other embodiments of the invention do not encompass a nucleic acid comprising any one of SEQ ID NO^{S}: 9928 to 9959. Some embodiments of the invention are not subject to these exclusions.

Immunoassays

The present invention utilizes various immunoassay techniques for identifying individuals exposed to SARSV and/or biological samples containing SARSV antigens or antibodies to SARSV.

Immunoassay Formats

The SARSV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with biological sample suspected of containing SARSV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen. Alternatively, anti-SARSV antibodies may be employed to detect the presence of SARSV antigens in a biological sample. Combination antigen/antibody assays are also contemplated; for example, as described for HCV detection in US patent 6,630,298.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (*e.g.*, in membrane or microtiter well form), polyvinyl chloride (*e.g.*, in sheets or microtiter wells), polystyrene latex (*e.g.*, in beads or microtiter plates, polyvinylidine fluoride, diazotized paper, nylon membranes, microchips, high or low density biochips, recombinant immunoassays (RIBA), microfluidity devices, micromagnetic beads, activated beads, and Protein A beads. For example, Dynatech Immunlon or Immunlon 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogenous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of SARSV antibodies in the antibody-antigen complexes is directly monitored. This may be accomplished by determining whether labeled anti-xenogeneic (*e.g.*, anti-human) antibodies which recognize an epitope on anti-SARSV antibodies will bind due to complex formation. In a competitive format, the amount of SARSV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-SARSV antibody (or in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled SARSV antibodies in the complex may be detected using a conjugate of antixenogeneic Ig complexed with a label, (*e.g.*, an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the SARSV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-SARSV antibody is present in the test specimen, no visible precipitate is formed.

There are at least three specific types of particle agglutination (PA) assays. These assays are used for the detection of antibodies to various antigens when coated to a support. One type of this assay is the hemagglutination assay using red blood cells (RBCs) that are sensitized by passively adsorbing antigen (or antibody) to the RBC. The addition of specific antigen antibodies present in the body component, if any, causes the RBCs coated with the purified antigen to agglutinate.

To eliminate potential non-specific reactions in the hemagglutination assay, two artificial carriers may be used instead of RBC in the PA. The most common of these are latex particles. However, gelatin particles may also be used. The assays utilizing either of these carriers are based on passive agglutination of the particles coated with purified antigens.

The SARSV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the native SARSV antigen, control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (*e.g.*, enzyme substrate) if the label does not generate a signal directly. The native SARSV antigen may be already bound to a solid matrix or separate with reagents for binding it to the matrix. Instructions (*e.g.*, written, tape, CD-ROM, *etc.*) for carrying out the assay usually will be included in the kit.

Immunoassays that utilize the native SARSV antigen are additionally useful in screening blood for the preparation of a supply from which potentially infective SARSV is lacking. The method for the preparation of the blood supply comprises the following steps. Reacting a body component, preferably blood or a blood component, from the individual donating blood with native SARSV antigen to allow an immunological reaction between SARSV antibodies, if any, and the SARSV antigen. Detecting whether anti-SARSV antibody--SARSV antigen complexes are formed as a result of the reacting. Blood contributed to the blood supply is from donors that do not exhibit antibodies to the native SARSV antigens.

Production of Antibodies

As explained above, the assay may utilize various antibodies which may be bound to a solid support, and that detect antigen or antigen/antibody complexes formed when SARSV infection is present in the sample. These antibodies may be polyclonal or monoclonal antibody preparations, monospecific antisera, human antibodies, or may be hybrid or chimeric antibodies, such as humanized antibodies, altered antibodies, $F(ab')_2$ fragments, F(ab) fragments, Fv fragments, single-domain antibodies, dimeric or trimeric antibody fragment constructs, minibodies, or functional fragments thereof which bind to the antigen in question.

Antibodies are produced using techniques well known to those of skill in the art and disclosed in, for example, US Pat. Nos. 4,011,308; 4,722,890; 4,016,043; 3,876,504; 3,770,380; and 4,372,745. For example, polyclonal antibodies are generated by immunizing a suitable animal, such as a mouse, rat, rabbit, sheep or goat, with an antigen of interest. In order to enhance immunogenicity, the antigen can be linked to a carrier prior to immunization. Such carriers are well known to those of ordinary skill in the art. Immunization is generally performed by mixing or emulsifying the antigen in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). The animal is generally boosted 2-6 weeks later with one or more injections

of the antigen in saline, preferably using Freund's incomplete adjuvant. Antibodies may also be generated by *in vitro* immunization, using methods known in the art. Polyclonal antiserum is then obtained from the immunized animal.

Monoclonal antibodies are generally prepared using the method of Kohler & Milstein (1975) *Nature* 256:495-497, or a modification thereof, as described above.

As explained above, antibody fragments which retain the ability to recognize the antigen of interest, will also find use in the subject immunoassays. A number of antibody fragments are known in the art which comprise antigen-binding sites capable of exhibiting immunological binding properties of an intact antibody molecule. For example, functional antibody fragments can be produced by cleaving a constant region, not responsible for antigen binding, from the antibody molecule, using *e.g.*, pepsin, to produce $F(ab')_2$ fragments. These fragments will contain two antigen binding sites, but lack a portion of the constant region from each of the heavy chains. Similarly, if desired, Fab fragments, comprising a single antigen binding site, can be produced, *e.g.*, by digestion of polyclonal or monoclonal antibodies with papain. Functional fragments, including only the variable regions of the heavy and light chains, can also be produced, using standard techniques such as recombinant production or preferential proteolytic cleavage of immunoglobulin molecules. These fragments are known as Fv. See, *e.g.*, Inbar *et al.* (1972) Proc. Nat. Acad. Sci. USA 69:2659-2662; Hochman *et al.* (1976) Biochem 15:2706-2710; and Ehrlich *et al.* (1980) Biochem 19:4091-4096.

A single-chain Fv ("sFv" or "scFv") polypeptide is a covalently linked V_H-V_L heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes linked by a peptide-encoding linker. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85:5879-5883. A number of methods have been described to discern and develop chemical structures (linkers) for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, US Pat. Nos. 5,091,513, 5,132,405 and 4,946,778. The sFv molecules may be produced using methods described in the art. See, *e.g.*, Huston *et al.* (1988) Proc. Nat. Acad. Sci. USA 85:5879-5883; US Pat. Nos. 5,091,513, 5,132,405 and 4,946,778. Design criteria include determining the appropriate length to span the distance between the C-terminus of one chain and the N-terminus of the other, wherein the linker is generally formed from small hydrophilic amino acid residues that do not tend to coil or form secondary structures. Such methods have been described in the art. See, *e.g.*, US Pat. Nos. 5,091,513, 5,132,405 and 4,946,778. Suitable linkers generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility.

"Mini-antibodies" or "minibodies" will also find use with the present invention. Minibodies are sFv polypeptide chains which include oligomerization domains at their C-termini, separated from the sFv by a hinge region. Pack *et al.* (1992) Biochem 31:1579-1584. The oligomerization domain comprises self-associating a-helices, *e.g.*, leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to facilitate in vivo folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, *e.g.*, Pack *et al.* (1992) Biochem 31:1579-1584; Cumber *et al.* (1992) J. Immunology 149B: 120-126.

Production of SARS Antigens

The SARSV antigens used in the present invention are generally produced recombinantly. Thus, polynucleotides encoding SARSV antigens for use with the present invention can be made using standard techniques of molecular biology. For example, polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. Furthermore, the desired gene can be isolated directly from viral nucleic acid molecules, using techniques described in the art, such as those described for HCV in Houghton *et al.*, US Pat. No. 5,350,671. The gene encoding the antigen of interest can also be produced synthetically, rather than cloned. The molecules can be designed with appropriate codons for the particular sequence (preferably optimum codons for the expression host of choice). The complete sequence is then assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, *e.g.*, Edge (1981) *Nature* 292:756; Nambair *et al.* (1984) *Science* 223:1299; and Jay *et al.* (1984) *J. Biol. Chem.* 259:6311.

Thus, particular nucleotide sequences can be obtained from vectors harboring the desired sequences or synthesized completely or in part using various oligonucleotide synthesis techniques known in the art, such as site-directed mutagenesis and polymerase chain reaction (PCR) techniques where appropriate. See, *e.g.*, Sambrook, *supra*. In particular, one method of obtaining nucleotide sequences encoding the desired sequences is by annealing complementary sets of overlapping synthetic oligonucleotides produced in a conventional, automated polynucleotide synthesizer, followed by ligation with an appropriate DNA ligase and amplification of the ligated nucleotide sequence via PCR. See, *e.g.*, Jayaraman *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:4084-4088. Additionally, oligonucleotide directed synthesis (Jones *et al.* (1986) *Nature* 54:75-82), oligonucleotide directed mutagenesis of pre-existing nucleotide regions (Riechmann *et al.* (1988) *Nature* 332:323-327 and Verhoeyen *et al.* (1988)

Science 239:1534-1536), and enzymatic filling-in of gapped oligonucleotides using T4 DNA polymerase (Queen *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:10029-10033) can be used under the invention to provide molecules having altered or enhanced antigen-binding capabilities, and/or reduced immunogenicity.

Once coding sequences have been prepared or isolated, such sequences can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Suitable vectors include, but are not limited to, plasmids, phages, transposons, cosmids, chromosomes (including artificial chromosomes, such as BACs or YACs) or viruses which are capable of replication when associated with the proper control elements.

The coding sequence is then placed under the control of suitable control elements, depending on the system to be used for expression. Thus, the coding sequence can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator, so that the DNA sequence of interest is transcribed into RNA by a suitable transformant. The coding sequence may or may not contain a signal peptide or leader sequence which can later be removed by the host in post-translational processing. See, *e.g.*, US Pat. Nos. 4,431,739; 4,425,437; 4,338,397.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector. For example, enhancer elements may be used herein to increase expression levels of the constructs. Examples include the SV40 early gene enhancer (Dijkema *et al.* (1985) *EMBO J.* 4:761), the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:6777) and elements derived from human CMV (Boshart *et al.* (1985) *Cell* 41:521), such as elements included in the CMV intron A sequence (US Pat. No. 5,688,688). The expression cassette may further include an origin of replication for autonomous replication in a suitable host cell, one or more selectable markers, one or more restriction sites, a potential for high copy number and a strong promoter.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (*i.e.*, RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the

sequences encoding the molecule of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it can be attached to the control sequences in the appropriate orientation; *i.e.*, to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

As explained above, it may also be desirable to produce mutants or analogs of the antigen of interest. Methods for doing so are described in, *e.g.*, Dasmahapatra *et al.*, US Pat. No. 5,843,752 and Zhang *et al.*, US Pat. No. 5,990,276. Mutants or analogs of SARSV proteins for use in the subject assays may be prepared by the deletion of a portion of the sequence encoding the polypeptide of interest, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, and the like, are well known to those skilled in the art. See, *e.g.*, Sambrook *et al.*, *supra*; Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* (1985) 82:448; Geisselsoder *et al.* (1987) *BioTechniques* 5:786; Zoller & Smith (1983) *Methods Enzymol.* 100:468; Dalbie-McFarland *et al.* (1982) *Proc. Natl. Acad. Sci USA* 79:6409.

The molecules can be expressed in a wide variety of systems, including insect, mammalian, bacterial, viral and yeast expression systems, all well known in the art.

For example, insect cell expression systems, such as baculovirus systems, are known to those of skill in the art and described in, *e.g.*, Summers & Smith, *Texas Agricultural Experiment Station Bulletin* No. 1555 (1987). Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego Calif. ("MaxBac" kit). Similarly, bacterial and mammalian cell expression systems are well known in the art and described in, *e.g.*, Sambrook *et al.*, *supra*. Yeast expression systems are also known in the art and described in, *e.g.*, *Yeast Genetic Engineering* (Barr *et al.*, eds., 1989) Butterworths, London.

A number of appropriate host cells for use with the above systems are also known. For example, mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human embryonic kidney cells, human hepatocellular carcinoma cells (*e.g.*, Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Similarly, bacterial hosts such as *E.coli*, *Bacillus subtilis*, and *Streptococcus* spp., will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*,

Kluyveromyces lactis, Pichia guillerimondii, Pichia pastoris, Schizosaccharomyces pombe and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia, Aedes aegypti, Autographa californica, Bombyx mori, Drosophila melanogaster, Spodoptera frugiperda, and Trichoplusia ni.*

Nucleic acid molecules comprising nucleotide sequences of interest can be stably integrated into a host cell genome or maintained on a stable episomal element in a suitable host cell using various gene delivery techniques well known in the art. See, *e.g.*, US Pat. No. 5,399,346.

Depending on the expression system and host selected, the molecules are produced by growing host cells transformed by an expression vector described above under conditions whereby the protein is expressed. The expressed protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth media, the product can be purified directly from the media. If it is not secreted, it can be isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

EXAMPLE

For useful expression of SARSV antigens in *Saccharomyces cerevisiae* and *Pichia pastoris*, insect cells, and mammalian cells, the following domains are cloned into expression vectors as listed in the Table below. The nt sequence numbers are from the SARSV sequence of SEQ ID NO: 1.

- RNA polymerase 1a: SARS nt 250-13398
- RNA polymerase 1b: SARS nt 13399-21470
- ORFns.envelope (homologous to ns2, hemagglutinin-esterase envelope glycoprotein, and spike glycoprotein): SARS nt 21477-25244
- Membrane: SARS nt 27849 - 28103
- Nucleocapsid: SARS nt 28105 - 29373

A combination of PCR and synthetic oligos is used to create the above domains with restriction sites tailored to the following expression vectors:

Restriction ends	Vector	Promoter	Expression host
*Hind*III/*Sal*I	pBS24.1	ADH2/GAPDH	AD3/Saccharomyces
*Eco*RI/*Sal*I	pBS24.1	ADH2/GAPDH/SOD fusion	AD3/Saccharomyces
XbaI/*Sal*I	pAO815	AOXI	GS115/Pichia pastoris
*Eco*RI/*Bam*HI	pCMVkm2	CMVp/Enhancer/IntronA	HVK-293/Transient transfection
*Eco*RI/*Xma*I	pCMVIII	CMVp/Enhancer/IntronA	CHO stable cell line
*Nhe*I/*Sal*I	pBluBac4.5	Polyhedrin	Cell lines employed by Chiron include: Sf9, Sf21, Tn5

IV. TREATMENT OF SARS INFECTION WITH RNAi

RNA interference or "RNAi" is a term initially coined by Fire and co-workers to describe the observation that double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Fire *et al.*, Nature 391, 806-811(1998)). RNAi most likely involves mRNA degradation, resulting in sequence-specific, post-transcriptional gene silencing in many organisms. RNAi is a post-transcriptional process triggered by the introduction of double-stranded RNA which leads to gene silencing in a sequence-specific manner. RNAi has been reported to occur naturally in organisms as diverse as nematodes, trypanosmes, plants and fungi. It most likely serves to protect organisms from viruses, modulate transposon activity and eliminate aberrant transcription products.

The first evidence that dsRNA could achieve efficient gene silencing through RNAi came from studies on the nematode *Caenorhabditis elegans* (Fire *et al.* (1998) *Nature*, 391:806-811 and US Patent No. 6,506,559). Later studies in the fruit fly *Drosophila melanogaster* demonstrated that RNAi is a two-step mechanism (Elbashir *et al.* (2001) *Genes Dev.*, 15(2): 188-200). First, long dsRNAs are cleaved by an enzyme known as Dicer in 21-23 nucleotides (nt) fragments, called small interfering RNAs (siRNAs). Then, siRNAs associate with a ribonuclease complex (termed RISC for RNA Induced Silencing Complex) which target this complex to complementary mRNAs. RISC then cleaves the targeted mRNAs opposite the complementary siRNA, which makes the mRNA susceptible to other RNA degradation pathways.

RNAi is the phenomenon where dsRNA corresponding to a targeted DNA or RNA sequence can suppress or silence gene expression. Even though dsRNA can mediate gene-specific interference in mammalian cells in some circumstances (Wianny & Zernicka-Goetz (2000) *Nature Cell Biol.* 2:70-75; Svoboda *et al.* (2000) *Development* 17:4147-4156) the use of RNAi in mammalian somatic cells is often limited due to the dsRNA triggering dsRNA-dependent protein kinase (PKR) which in turn inactivates translation factor eIF2a and causes a generalized suppression of protein synthesis and often times apoptosis (Gil & Esteban (2000) *Apoptosis* 5:107-114).

Recently, gene-specific suppression using siRNA of approximately 21 or 22 base pairs in length, corresponding to targeted RNA or DNA sequences, were shown to disrupt the expression of these targeted sequences in mammalian cells (Elbashir, S.M., *et al.*, *Nature* 411: 494-498 (2001)). However, it is not clear that all RNA or DNA sequences of a mammalian cell's genome are susceptible to siRNA. It is also uncertain that every mammalian cell type possesses the necessary machinery for effecting gene-specific suppression using siRNA. Further, siRNA is of limited use for at least two reasons: the transient nature of the suppression effect seen in cells where the siRNA has been administered; and in some instances the necessity for chemical synthesis of siRNAs before their use (Tuschl T., *Nature Biotechnol.*, 20: 446-448 (2002)). Also

the instability of these short, synthetic RNAs makes it presents problems for any long term use of these siRNAs a pharmaceutical.

To overcome this limitation, the present invention provides a modified siRNA with increased stability against nuclease degradation while still maintaining its ability to inhibit viral replication via RNA interference. Such modification to the ribonucleotides in the siRNAs, adds a chemical group via chemical synthesis or *in vitro* transcription or longer modified RNAs can be prepared by either of these methods and cut into siRNAs using Dicer.

Although other methods for gene-specific suppression have utilized chemically-modified nucleic acids, such as antisense and ribozyme technology, such modification destroys critical enzymatic activities necessary for the function of these technologies. In regard to antisense technology, modification of the ribonucleotides destroys RNaseH activity, whereas such modification abolishes the catalytic activity of ribozymes.

The present invention provides a double-stranded RNA (dsRNA) molecule modified for protection against nuclease degradation with a length from about 10 to about 30 nucleotides which is able to inactivate a virus in a mammalian cell. The invention also provides a method of inactivating a virus by administering modified small interfering RNAs (siRNAs) that are modified so that they are nuclease or RNase resistant and retain the biological activity of being able to inhibit viral replication by targeting a RNA sequence in a virus.

The invention is further directed to a method of making modified siRNAs that target a RNA sequence in a virus comprising preparing a modified-double stranded RNA (dsRNA) fragment containing at least one modified ribonucleotide in at least one strand that spans the genome of the virus; and cleaving the modified-dsRNA fragments with recombinant human Dicer resulting in more than one modified siRNA.

The present invention provides a modified dsRNA molecule of from about 10 to about 30 nucleotides which mediates targeted RNA interference in hepatic or SARS-infected cells.

As used herein RNA interference, or RNAi, is used to mean sequence-specific, or gene specific, suppression of gene expression (protein synthesis), without causing a generalized suppression of protein synthesis in cells harboring the siRNA. The invention is not limited to a particular theory of the mechanism of action of RNAi. For example, RNAi may involve degradation of messenger RNA (mRNA) in an RNA-induced silencing complex (RISC), preventing translation of the transcribed mRNA, or it may involve the methylation of genomic DNA, shunting transcription of the gene. The lack of gene expression caused by RNAi may be transient, lasting a short period of time, or it may be stable, or permanent, lasting an indefinite period of time.

The term RNA is meant as is recognized in the art. Further, as used herein, RNA is used to mean double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA) or a dsRNA with a

single-stranded overhang. dsRNAs within the meaning of the present invention includes short interfering RNA (siRNA), micro RNA (miRNA) and small hairpin RNA (shRNA), Additionally, RNA is also used to mean messenger RNA (mRNA), transfer RNA (tRNA) or ribosomal RNA (rRNA).

The present invention is directed to small interfering RNA (siRNA) which have been chemically modified to confer increased stability against nuclease degradation yet these siRNAs are still able to bind to target RNAs, that may be present in a cells. In the case where the target RNA is a virus specific RNA, the modified siRNAs are able to bind to the virus specific RNAs and inactivate the virus. A modified siRNA of the present invention comprises a modified ribonucleotide, wherein the siRNA is resistant to enzymatic degradation, such as RNase degradation, and yet retains the ability to inhibit viral replication. The modified siRNA is more specifically modified at the 2' position of the ribose in the siRNA. The modification is at the 2' position of at least one ribonucleotide of said siRNA. Attachment of receptor-binding ligands to siRNA molecules can be used to target the siRNA to a desired cell type. For example, attachment of cholesterol at the 5'-end or 3'-end of the siRNA molecule, to give a cholesteryl siRNA, can enhance targeting to hepatocytes. Other ligands for receptor mediated siRNA targeting to liver include HBV surface antigen, LDL, and others.

More specifically, the siRNA is modified at at least one pyrimidine, at least one purine or a combination thereof. However, generally all pyrimidines, or all purines or a combination of all pyrimidines and all purines of the siRNA are modified. More preferably, the pyrimidines are modified and these pyrimidines are cytosine, a derivative of cytosine, uracil, a derivative of uracil or a combination thereof. It also is contemplated to modify the selected ribonucleotides in at least one strand of the siRNA or the ribonucleotides in both strands of the siRNA are modified.

The nucleotides containing pyrimidine bases found in RNA (cytidine and uridine) can be chemically modified by adding any molecule that inhibits RNA degradation or breakdown to the 2' position of the ribose molecule. The 2'-modified pyrimidine nucleotide can be formed using a number of different methods. The 2' modification confers increased stability to the siRNA by making the siRNA impervious or resistant to nuclease activity. Thus, the 2' modified siRNA has a longer serum half-life and is resistant to degradation compared to unmodified siRNA. The siRNA also may be modified completely or partially.

Regarding chemical modification of siRNAs, a molecule from the halide chemical group is preferably added to the ribonucleotide of the siRNA. Within the halides, fluorine is the preferred molecule but other chemical molecules, in addition to fluoro-, such as methyl-, methoxyethyl- and propyl-modifications can also we made. But the preferred modications is fluoro-modification, such as a 2'-fluoro-modication or a 2',2'-fluoro-modification.Thus, in a preferred

embodiment of the invention, the siRNA is modified by adding a fluorine molecule to the 2' carbon of the pyrimidine ribonucleotide. The siRNA may be fluorinated completely or partially. For example, only the cytosine nucleotides need be fluorinated. Alternatively, only the uracil nucleotide need be fluorinated but both uracil and cytosine can be fluorinated. Furthermore, only one strand, either sense or antisense, of the siRNA can be fluorinated. Even partial 2' fluorination the siRNA gives protection against nucleolytic degradation. Furthermore, it is important to note the 2' fluorinated siRNA is not toxic to cells, an unexpected result given that fluorine chemistry usually is toxic to living organisms.

The siRNA of the present invention is designed to interact with a target nucleotide sequence. Most preferably this target nucleotide sequence is a disease producing agent or pathogen of which one wishes to inhibit gene expression. More preferably, this target nucleotide sequence is in a virus genome, and further this virus genome is from a RNA virus or a DNA virus is selected from the group consisting of hepatitis C virus (HCV), hepatitis A virus, hepatitis B virus, hepatitis D virus, hepatitis E virus, Ebola virus, influenza virus, rotavirus, reovirus, retrovirus, poliovirus, human papilloma virus (HPV), metapneumovirus and coronavirus. The most preferred virus is SARS virus.

Modfied siRNA may be prepared in a number of ways, such as by chemical synthesis, T7 polymerase transcription, or by treating modified long double stranded RNA (dsRNA) prepared by one of the two previous methods with Dicer enzyme. Dicer enzyme can be used to cleave dsRNA that is about 500 base pairs to about 1000 base pairs in size, to created mixed populations of dsRNA from about 21 to about 23 base pairs in length. Furthermore, an unexpected result of using the Dicer enzyme method is that Dicer enzyme will cleave modified strands of dsRNA, such as 2' fluorinated modified dsRNA. Before development of this method, it was previously thought that Dicer would not be able to cleave modified siRNA. The Dicer method can be carried out using the Dicer siRNA Generation Kit available from Gene Therapy Systems, San Diego, CA.

As used herein, small interfering RNA (siRNA) is defined as double- or single-stranded RNA of from about 10 to about 30 nucleotides in length, more preferably 12-28 nucleotides, more preferably 15-25 nucleotides, even more preferably 19-23 nucleotides and most preferably 21-23 nucleotides. The length of a siRNA as used herein, is determined by the length of one of the strands of the RNA. For example, a siRNA that is described as 21 nucleotides long (a 21-mer) may comprise two opposite strands of RNA which anneal together for 19 contiguous base pairings. The two remaining nucleotides on one end of the molecule would not anneal to the opposite strand, thus creating an "overhang". The overhang can be at the 5' or the 3' end of the dsRNA. Preferably, the overhang is at the 3' end of the RNA strand. The length of a double-stranded RNA where the two opposite strands are not the same length will be designated by the

longer of the two strands. For example, a dsRNA comprising one strand which is 21 nucleotides long and anneals to an opposite strand that is 20 nucleotides long, will be considered, as used herein, a 21-mer.

Preferably, the siRNA of the present invention will comprise a 3' overhang of about 2 to 4 bases. More preferably, the 3' overhang is 2 nucleotides long. Even more preferably, the 2 nucleotides comprising the 3' overhang are uridine (U).

In one embodiment, the invention provides an RNA molecule comprising a nucleotide sequence at least 80% identical to the nucleotide sequence of the target agent or virus. Preferably, the RNA molecule of the present invention is at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of the target agent or virus.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97% 98%, 99% or 100% identical to the nucleotide sequence of the target agent or virus can be determined conventionally using known computer programs such as the *Bestfit* program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). *Bestfit* uses the local homology algorithm of Smith & Waterman (*Advances in Applied Mathematics* 2:482-489 (1981)) to find the best segment of homology between two sequences. When using *Bestfit* or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present invention provides a method of inactivating a target agent or preferably a virus in a patient comprising administering to the patient a modified siRNA in an effective amount to inactivate the targeted agent or virus. RNA interference towards a targeted DNA segment in a cell can be achieved by administering a dsRNA molecule or siRNA to the cells, wherein the nucleotide sequence of the dsRNA molecule corresponds to the nucleotide sequence of the targeted DNA segment. Preferably, the RNA molecule used to induce targeted RNAi is siRNA.

Gene suppression, targeted suppression, sequence-specific suppression, targeted RNAi or sequence-specific RNAi are used interchangeably herein. Furthermore, sequence-specific suppression, as used herein, is determined by separately assaying the levels of the protein targeted for suppression in cells containing the siRNA (experimental cells) and in cells not containing the identical siRNA (control cells), and comparing the two values. Furthermore, the experimental and control cells must be derived from the same source and same animal. For example, the control and experimental cells can be, but are not limited to, normal human hepatic cells as cell culture *in vitro*, or they can derived from a hepatocellular carcinoma. Further, the

control and experimental cells used in determining the level or quantity of gene suppression must be assayed under similar, if not identical, conditions.

As used herein the phrase "targeted DNA segment" is used to mean a DNA sequence encoding, in whole or in part, an mRNA for a targeted protein, including introns or exons, where suppression is desired. DNA segment can also mean a DNA sequence that normally regulates expression of the targeted protein, including but not limited to the promoter of the targeted protein. Furthermore, the DNA segment may or may not be a part of the cell's genome or it may be extrachromosomal, such as plasmid DNA.

The present invention is further directed to inactivating a virus in a patient comprising administering to a patient a modified siRNA in an effective amount to inactivate the virus. The siRNA is preferably about 10 to about 30 nucleotides in length, more preferably 12-28 nucleotides, more preferably 15-25 nucleotides, even more preferably 19-23 nucleotides and most preferably 21-23 nucleotides. The method preferably utilizes a 2' modified siRNA that is modified at the 2' position of at least one ribonucleotide of said siRNA. The method utilizes a siRNA that is modified with chemical groups selected from the group consisting of fluoro-, methyl-, methoxyethyl- and propyl-modification. The fluoro-modification is preferred and either a 2'-fluoro-modication or a 2',2'-fluoro-modification is useful in the present invention and preferred.

The modification may be at the pyrimidines, the purines or a combination thereof of the siRNA are modified. More preferably the pyrimidines are modified, such as cytosine, a derivative of cytosine, uracil, a derivative of uracil or a combination thereof. In one embodiment, at least one strand of the siRNA contains at least one modified nucleotide and in an alternate embodiment, oth strands of the siRNA contains at least one modified nucleotide.

The method is intended to target disease causing agents or pathogens, an more particularly viruses, which can be either a RNA virus or a DNA virus, which are selected from the group consisting of hepatitis C virus (HCV), hepatitis A virus, hepatitis B virus, hepatitis D virus, hepatitis E virus, Ebola virus, influenza virus, rotavirus, reovirus, retrovirus, poliovirus, human papilloma virus (HPV), metapneumovirus and coronavirus. More preferably the target virus is a SARS virus. The present method utilizes a siRNA prepared by (a) identifying a target nucleotide sequence in a virus genome, preferably SARS virus, for designing a small interfering RNA (siRNA); and (b) producing a siRNA that has been modified to contain at least one modified nucleotide. More preferably, the siRNA comprises a dsRNA molecule with a first strand ribonucleotide sequence corresponding to a nucleotide sequence corresponding to a target nucleotide sequence in said virus and a second strand comprising a ribonucleotide sequence complementary to said target nucleotide sequence, wherein said first and second strands are separate complementary strands that hybridize to each other to form said dsRNA molecule, and

further wherein the first strand ribonucleotide sequence, the second strand ribonucleotide sequence or both the first and second strand ribonucletide sequences comprise at least one modified nucleotide. In this method, the target nucleotide sequence comprises a conserved nucleotide sequence necessary for SARS virus replication, and the conserved nucleotide sequence is selected from the group consisting of SEQ ID NO: 7292, SEQ ID NO: 7293, SEQ ID NO: 7294, SEQ ID NO: 7295, SEQ ID NO: 7296, SEQ ID NO: 7297, SEQ ID NO: 7298, SEQ ID NO: 7299, SEQ ID NO: 7300 and SEQ ID NO: 7301. Preferably, the nucleotide sequence is selected from the group consisting of SEQ ID NO: 7292 and SEQ ID NO: 7293. Still more preferably, the nucleotide sequence is SEQ ID NO: 7293.

The siRNA disclosed in this application may be prepared with modified ribonucleotides as described herein. Further, the modified ribonucleotide of the siRNA used in the present method is incorporated into said siRNA by chemical synthesis or enzymatic synthesis.

The siRNA disclosed in this application may or may not have a 5' triphosphate group.

The modified siRNA is administered to a patient by a method selected from the group consisting of intravenous injection, subcutaneous injection, oral delivery, and liposome delivery. The modified siRNA accumulates in an organ, tissue or body system of the patient that are the liver, gastrointestinal tract, respiratory tract, cervix or skin.

The present invention also provides a method of inhibiting the replication of a virus, such as SARS virus, in cells positive for SARS virus comprising transfecting SARS-positive cells with a vector that directs the expression of modified siRNA which is specific for SARS. The cells are evaluated to determine if a marker in the cells has been inhibited by the modified siRNA.

The term patient, as used herein, can be an animal, preferably a mammal. More preferably the subject can be a primate, including non-human and humans. The terms subject and patient can be used interchangeably.

The treatment envisioned by the current invention can be used for subjects with a pre-existing viral infection, or for subjects pre-disposed to a SARS virus infection. Additionally, the method of the current invention can be used to correct or compensate for cellular or physiological abnormalities involved in conferring susceptibility to viral infections in patients, and/or to alleviate symptoms of a viral infection in patients, or as a preventative measure in patients.

The method of treating a patient having a viral infection involves administration of compositions to the subjects. As used herein, composition can mean a pure compound, agent or substance or a mixture of two or more compounds, agents or substances. As used herein, the term agent, substance or compound is intended to mean a protein, nucleic acid, carbohydrate, lipid, polymer or a small molecule, such as a drug.

In one embodiment of the current invention, the composition administered to the subject is a pharmaceutical composition. Further, the pharmaceutical composition can be administered orally, nasally, parenterally, intrasystemically, intraperitoneally, topically (as by drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral," as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. The pharmaceutical compositions as contemplated by the current invention may also include a pharmaceutically acceptable carrier.

By "pharmaceutically acceptable carrier" is intended, but not limited to, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type, such as liposomes.

A pharmaceutical composition of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compositions of the present invention can also contain adjuvants such as, but not limited to, preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, to prolong the effect of the drugs, it is desirable to slow the absorption from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, can depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be

controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include, but are not limited to, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compounds are mixed with at least one item pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, acetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form can also comprise buffering agents.

Solid compositions of a similar type can also be employed as fillers in soft and hard filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They can optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms can contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate,

propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, can contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Alternatively, the composition can be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition is preferably such that the active ingredients do not dissolve therein to any substantial extent. The pressurized composition can also contain a surface active agent. The surface active agent can be a liquid or solid non-ionic surface active agent or can be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

The compositions of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to the compounds of the invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art (*see*, for example, Prescott, Ed., *Meth. Cell Biol. 14*:33 *et seq* (1976)).

One of ordinary skill will appreciate that effective amounts of the agents of the invention can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. The agents can be administered to a subject, in need of treatment of viral infection, as pharmaceutical compositions in combination with one or more pharmaceutically acceptable excipients. It will be understood that, when administered to a human patient, the total daily usage of the agents or composition of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or composition employed; the specific agents or composition employed; the age, body weight, general health, sex and diet of the patient; the time

of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the agents at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosages until the desired effect is achieved.

Dosing can also be arranged in a patient specific manner to provide a predetermined concentration of the agents in the blood, as determined by techniques accepted and routine in the art. Thus patient dosaging can be adjusted to achieve regular on-going blood levels, as measured by HPLC, on the order of from 50 to 1000 ng/ml.

It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of the invention or any embodiment thereof.

The modified siRNA is prepared by custom chemical synthesis by Dharmacon, at Lafayette CO. Each C and U within the siRNA duplex (GL2), has been substituted with 2'-F-U and 2'-F-C except for the 3'-end overhang, which was dTdT.

To test the stability of 2' chemically modified siRNA compared to unmodified siRNA (siRNA), the following experiment is performed. 4ngs of siRNA are added to a 20 μL volume of 80% human serum from a healthy donor. This mixture is incubated at 37°C for various times ranging from 1 minute up to 10 days. The same process is performed for 2' fluorine modified siRNA (2'-F siRNA). When the incubation process is finished, the mixtures are placed on ice and then immediately separated by PAGE along with a ^{32}P-siRNA control. The 2' modified siRNA is stable as compared to unmodified siRNA.

V. IDENTIFICATION OF THERAPEUTICALLY ACTIVE AGENTS FOR TREATMENT OF SARS VIRUS INFECTION

The invention provides methods for treating SARS by administering therapeutically active agents, such as small molecule compounds, to a mammal, as well as methods of identifying therapeutically active agents, such as potent small molecules, for the treatment of SARS virus infection.

In one aspect of the invention a method of identifying a therapeutically active agent is provided comprising: (a) contacting the therapeutically active agent with a cell infected with the SARS virus; (b) measuring attenuation of a SARS related enzyme.

In a more particular embodiment, the therapeutically active agent is a small molecule. In another more particular embodiment, the therapeutically active agent is a nucleoside analog (*e.g.* Ribavirin). In another more particular embodiment the small molecule is a SMIP or peptidic immunomodulating compound. In another more particular embodiment the therapeutically

active agent is a peptoid, oligopeptide, or polypeptide. In another embodiment the SARS related enzyme is SARS protease. In another embodiment the SARS related enzyme is SARS polymerase. In still another embodiment the SARS related enzyme is a kinase. In still another embodiment, the SARS related enzyme is a protease. The furin inhibitor peptidyl chloromethylketone prevents blocks cell-cell fusion after MHV infection (de Haan *et al.* (2004) *J Virol*), which offers guidance for SARS therapy.

The invention includes a cell-based assay that can be used to screen for and identify a therapeutically active agent for the treatment of SARS virus infection. Therapeutically active agents of the invention include agents that inhibit, prevent or reduce the replication of a SARS virus. Such agents can be identified by infecting a cultured cell (such as, for example, VERO cells) with a SARS virus and evaluating the impact of potential antiviral compounds on SARS virus replication. Assays to measure the effect of a potential antiviral compound on virus replication are known in the art and may be based on a variety of parameters.

The cell-based assay may be used in a high-throughput screen to identify therapeutically active compounds from chemical libraries comprising potential antiviral compounds. Therapeutically active compounds suitable for use in the invention may inhibit any SARS viral target that is essential for viral replication in whole cells. Efficacy (the ability of a compound to inhibit or inactivate the target, be it viral or cellular, that results in the reduction of virus in the culture) of the therapeutic agent is measured by assessing the viability and/or the proliferation of surviving cells in a SARS virus infected cell culture.

A number of methods can be used to measure cell viability are known in the art, such as assays measuring cellular enzymes, proteins, nucleotide triphoshates (*e.g.* ATP), nucleic acids (*e.g.* host cell mRNA (*e.g.* GAPDH) or rRNA sequences) or cellular metabolites such as MTT or MTS. In addition, fluorescent (including, for example HSV paper) or non-fluorescent dyes (*e.g.* propidium diiodide) or labeling of DNA can be used to measure indications of cell viability and/or proliferation.

Alternatively, efficacy of a compound or sample can be determined by directly measuring the amount of virus or viral products in the culture. Methods for measuring the amount of virus, viral genome or viral products include: PCR, RT-PCR, TMA, reporter proteins with fluourescent or luminescent qualities or enzymatic functions (*e.g.*, luciferase, alkaline phosphatase, GFP) or proteins that can be detected by antibodies (*e.g.* EGF) that might be incorporated into the viral genome prior to infection of the cell culture. Further, viral products such as viral proteins can be measured by ELISA or enzymatic activities. Methods for identifying viral polynucleotides, viral proteins and antibodies specific to viral proteins are discussed above.

Potential antiviral compounds are applied to the cell-based assay at a concentration of approximately 10 μM and compound classes having therapeutic effect are identified by

measuring the parameter of choice (such as cell viability/proliferation or the virus or viral genome or a viral product be it viral in origin or non-virus in orgin). Once compounds are identified as having activity, they are resynthesized, and analoged. Starting with the identified compound, many analogs and new compounds are synthesized during consecutive optimization cycles of synthesis, biological profiling and modeling techniques to optimize the to the lead structure until *in vivo* activity is elucidated and optimized.

Cells suitable for use in the assay include the cells described above as suitable for vaccine production. Preferably, the cells are African green monkey kidney cells (Vero) cells. Human embyronic lung fibroblasts or normal human diploid fibroblasts may also be used in the invention.

In one embodiment, the invention includes a fluorescence based cytopathogenicity assay to measure the effect of a potential antiviral compound on a cell-based assay. One example of a fluorescence based cytopathogenicity assay is illustrated below.

1×10^4 Vero cells per well of a microtiter plate (MTP) are infected with a defined amount of SARS virus selected within the following ranges for optimal MOI: 5-10, 10-25, 25-50, 50-100, 100-500, or 500-1000 PFU in a total volume of 200 μl media (M199 medium supplemented with 5% FCS, 2 mM glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin) in the presence or absence of the potential antiviral compound and incubated for at least 1, 2, 3, 4, 5, 6, or 7 days at 37°C, 5% CO_2. The wells of the MTP are washed with PBS (200 μl) and then filled with 200 μl PBS containing 10 μg/ml fluorescein diacetate. After a 45-min incubation at room temperature, fluorescence is measured at 485 nm excitation and 538 nm emission wavelengths. IC_{50} values are determined by a nonlinear plot of antiviral activity as a function of drug concentration.

Other cell based assays are known in the art and include, among others, methods of GFP detection and Luc detection. In addition, a Promega kit is commercially available that provides additional methods of measuring cell viability, *etc.*

In one embodiment, the invention includes a method of measuring the efficacy of a potential antiviral compound using RT-PCR to detect the levels of SARS viral RNA in the cell based assay. Methods of using RT-PCR are known in the art. One example of such an assay is described below.

5×10^6 Vero cells are seeded in tissue culture. Flasks containing the cells are incubated over night at 37°C, 5% CO_2. The cells are infected (m.o.i. = 1) with SARS virus in the presence and absence of potential antiviral compounds. Optionally, the cells may be pretreated with the potential compound prior to infection. In either case, a suitable control cell assay is also prepared.

The RNA of infected cells is purified at 2 h (UL54), 12 h (UL8) and 16 h (UL13) after infection, (Qiagen) RNA purification (RNeasy kit; 40 μl elution) and quantified (absorption at 260 nm). The RNA (2 μg) is reverse transcribed with a specific primer (2 pmol, using one of the primer pairs described herein) into cDNA according to theSuperscript II protocol (Invitrogen). Aliquots (2 μl) of the reverse transcription reaction are amplified by PCR. Fragments of the appropriate target SARS gene, *i.e.*, a gene encoding a SARS enzyme, are amplified in 30 cycles (UL54 and UL8: 3 min, 94°C hot start; 1 min, 94°C denaturation; 1 min, 55°C annealing; 1 min, 72 °C polymerization. UL13: 3 min, 94 °C hot start; 1 min, 94 °C denaturation; 1 min, 60 °C annealing; 1 min, 72°C polymerization) by PCR (Taq-Polymerase, Stratagene), in a 100-μl reaction volume with the appropriate oligonucleotides, as described herein at 0.1 nmol each. 8-μl aliquots of cycle 20–30 (lanes 2–12) of the PCR were resolved on a 2% agarose gel (Invitrogen) according to the manufacturer's instructions.

Cell-based assays of the invention may optionally use a variant or derivative of a wild-type SARS virus that has reduced or attenuated virulence in humans and/or animal models (*e.g.*, mouse, non-human primate, *etc.*) Use of such attenuated SARS viruses in screening methods may reduce safety concerns and precautions that would otherwise be associated with the pathogenic nature of the SARS virus and may eliminate or reduce the need for the implementation of cumbersome high containment levels during performance of the assays and screening of compounds.

The invention includes an enzyme-based assay that can be used to screen for and identify a therapeutically active agent for the treatment of SARS virus infection.

An embodiment of the invention is an assay comprising contacting a known quantity of SARS protease in solution to a peptide containing a detectable marker and cleavage site for SARS protease, wherein SARS protease activity is monitored by measuring the intensity of the marker on the cleaved product.

In a more particular embodiment, a method of assaying for SARS protease is provided comprising contacting a sample solution containing SARS protease with a peptide containing a fluorescent donor, fluorescent quencher, and cleavage site for SARS protease, said peptide being detectable with a fluorometer when cleaved, wherein SARS protease activity is determined in the sample by the amount of fluorescence detected by the fluorometer.

Assays based on the direct measurement of SARS protease inhibition may be utilized for screening for SARS therapeutics. Protease for such assays such as 3C-like protease and papain-like protease may be isolated and purified for such assays as described in Seybert, *et al.*, J. Gen. Virol., 78:71-75, 1997, Ziebuhr, *et al.*, Adv. Exp. Med. Biol., 440:115-120, 1998, Sims, *et al.*, Adv. Exp. Med. Biol. 440:129-134, 1998, Ziebuhr, *et al.*, J. Virol., 73:177-185, 1999, Teng, *et al.*, J. Virol., 73:2658-2666, 1999, Herold, *et al.*, J. Biol. Chem. 274:14918-14925, 1999, and

Ziebuhr, *et al.*, J. Biol. Chem. 276:33220-33232, 2001. Furthermore, Example 30 describes a novel method of purifying SARS protease using column chromatography. Example 31 describes a continuous fluorescence resonance energy transfer (FRET) assay for measuring SARS protease activity. Protease enzyme based assays such as the FRET assay demonstrated in Example 31 are readily adapted for high-throughput screening and are used for screening candidate antiviral compounds. Performance of the protease enzymatic assay in the presence of a SARS protease inhibitor compound will show a decreased amount of fluorescence at a given time when compared to negative control assay containing no test compound on a non-inhibiting control compound. Such a method would involve the steps of: (a) providing an assay solution comprising SARS protease; (b) adding a test compound to the assay solution; (c) adding a substrate for SARS protease to the assay solution; and (d) measuring the proteolytic activity in the assay solution. In a preferred embodiment, the proteolytic activity is measured by the fluorescence of fluorophore product produced by the enzymatic activity of SARS protease.

Attenuated SARS virus variants generally contain one or more genome modifications or mutations (*e.g.*, substitutions, deletions, insertions) in protein encoding or non-coding regions. Specific examples of attenuating mutations include, for example, genetic modifications in the 5'-end noncoding region, leader sequence, intergenic regions, 3'-end noncoding region, ORF 1a, ORF 1b, S gene, E gene, M gene, N gene, or any of the nonstructural protein genes outside of the ORF 1a/1b region. Preferred attenuating mutations are in a SARS virus structural protein (*e.g.*, Spike (S)), a protease or polymerase domain, or a non-coding sequence (*e.g.*, 5'-end noncoding region, intergenic sequence). In addition, a cleavage site may be introduced or eliminated within the spike protein (see for example, Gombold *et al.*, J. Virol. 67:4504-4512, 1993; Bos *et al.*, Virology 214:453-463, 1995), such modification that may also be useful for optimization of expression of recombinant spike protein antigen (*e.g.*, for vaccine purposes).

A variety of methods are used according to the present invention in order to obtain attenuated variants of SARS virus. Such methods include serial passage of the SARS virus in cultured cells (*e.g.*, mammalian cell culture, such as fetal rhesus kidney cells or VERO cells), until the SARS virus demonstrates attenuated function. The serial propagation of virus may be performed at any temperature at which tissue culture passage attenuation occurs, and may be performed in conjunction with one or more steps of mutagenesis (*e.g.*, chemical mutagenesis). The attenuated phenotype of SARS virus variants, obtained after one or more cell culture passages, is readily measured by one skilled in the art. As used herein, attenuation refers to the decreased virulence of the SARS virus in a human subject. Evidence of attenuated function may be indicated by decreased levels of viral replication or by decreased virulence in an animal model.

Other methods of producing an attenuated SARS virus include cell culture passage of the virus at sub-optimal temperatures (cold passage), as well as introduction of attenuating mutations into the SARS viral genome by random mutagenesis (*e.g.*, chemical mutagenesis, such as using 5-fluorouracil) or using directed mutagenesis. Preparation and generation of attenuated RSV vaccines (the methods of which will generally applicable to SARS virus) are disclosed in, for example, EP 0640128, US Patent No. 6,284,254, US Patent No. 5,922,326, US Patent No. 5,882,651.

The number of passages required to obtain safe, immunizing attenuated virus is dependent at least in part on the conditions employed. Periodic testing of the SARS virus culture for virulence and immunizing ability in animals (*e.g.*, mouse, primate) can readily determine the parameters for a particular combination of tissue culture and temperature.

In another embodiment, the cell-based assay for screening of antiviral compounds is based on the readout of expression of a gene product (*e.g.*, reporter gene product) that is not from SARS virus. Gene products particularly suitable to the present invention include, but are not limited to those of the above-described assays.

In order to achieve such a read-out, the gene-of-interest (GOI) encoding said gene reporter gene product must be incorporated into a replicating SARS virus genome or construct derived from a SARS virus genome (*e.g.*, SARS virus replicon, SARS virus defective-interfering (DI) RNA). Figure 13 is a schematic depicting locations for incorporation of the reporter gene into a SARS virus genome. Preferably, insertion of a heterologous reporter gene-of-interest is at a site between existing SARS virus genes, such as for example, as shown in Figure 13. For example, the GOI may be inserted closely following the termination codon of a SARS virus gene (*e.g.*, ORF 1b, S, E, M, N). Insertion should be positioned in order to minimize disruption of mRNA transcription for the SARS virus gene(s). The GOI may also be inserted as an in-frame "fusion" with an existing SARS virus gene, such that sufficient function of the GOI is maintained for detection. To optimize expression, an additional SARS virus intergenic sequence (*e.g.*, SEQ ID NO: 7388, with or without additional flanking SARS virus sequences) may also be engineered into a position preceding the inserted GOI.

Incorporation of a GOI into SARS virus may be accomplished by one of skill in the art using a variety of techniques. For example, one preferred method is targeted RNA recombination, that takes advantage of the ability of coronavirus RNAs to undergo recombination within the cell (see for example Fischer *et al.*, J. Virol. 71:5148-5160, 1997; Koljesar *et al.*, J. Vet. Sci. 2:149-157, 2001). A construct of desired configuration (*e.g.*, cDNA of defective interfering RNA of SARS virus) containing the GOI flanked by SARS virus sequence (*e.g.*, intergenic sequence) is generated such that RNA may be transcribed directly within a eukaryotic cell or in vitro and transfected into susceptible cells also infected with SARS

virus. Recombinant virus containing the GOI is identified based on expression of the GOI encoded marker.

Alternatively, incorporation of a GOI into SARS virus may be accomplished by one of skill in the art by first assembling a full-length cDNA clone of the SARS virus, that can be used to produce infectious RNA transcripts *in vivo* (*e.g.*, from an RNA polymerase II promoter) or *in vitro* (*e.g.*, from a bacteriophage promoter). Although relatively long in genome length, such assembly of a full-length cDNA clone is now readily obtainable by one of skill in the art using standard molecular biology and reverse genetics techniques and the genome sequence of SARS virus (see for example, Thiel *et al.*, J. Gen. Virol., 82:1273-1281, 2001; Almazan *et al.*, Proc. Natl. Acad. Sci. USA 97:5516-5521, 2000; Thiel *et al.* (2003) *J Gen Virol* 82:1273-1281; Yount *et al* (2003) *PNAS USA* 100:12995-13000). Insertion of a heterologous GOI into a full-length SARS virus genome cDNA may be performed using a variety of techniques, such as for example, ligation into natural or synthetic restriction sites, PCR (*e.g.*, overlapping PCR), and recombination.

It may also be desirable to utilize similar SARS virus recombinants containing a gene-of-interest for antiviral screening, however, with further modification to minimize or eliminate virus-induced cytopathology (*e.g.*, CPE). Non-cytopathic derivatives from SARS virus may be obtained by one of skill in the art using a variety of methods. For example, a selectable marker (*e.g.*, drug resistance marker) may be incorporated as GOI into a SARS virus genome to produce infectious virus as described above (see for example, Perri *et al.*, J. Virol., 74:9802-9807, 2000). Infectious GOI-containing SARS virus or infectious genome RNA/cDNA is then used to infect/transfect cells (*e.g.*, VERO), with or without prior mutagenesis, after which time the infected cells are subjected to the appropriate selection. Only those cells containing SARS virus harboring both the selectable marker and one or more mutations rendering the virus non-cytopathic will survive the selection process and grow out. Active SARS virus replication in these cells is readily detected using a variety of detection techniques (*e.g.*, PCR, Northern blot) and such cells may serve as the substrate for cell-based screening assays. Mutations that result in the desired noncytopathic SARS virus phenotype may include nucleotide substitutions, deletions or additions, and may occur in a variety of genome coding or non-coding regions (*e.g.*, 5' or 3'-end noncoding regions, intergenic regions, ORF1a, ORF1b, a protease domain, a polymerase domain). The identification of such mutations is readily accomplished by exchange of sequences with wild-type (*e.g.*, parental) SARS virus and demonstrating transfer of the phenotype, and sequencing of the appropriate genome region. Similar mutations that reduce or eliminate cytopathogenicity also may be utilized in the context of a SARS virus derived replicon vector, either by similar selection directly using a SARS virus replicon or by specific engineering of the replicon based on mutation(s) identified in the context of infectious SARS virus as described

above. In addition, such mutations may serve as the basis for attenuated SARS virus derivatives, as described elsewhere in this document.

Alternatively, rather than using infectious SARS virus or its derivatives for cell-based screening assays, propagation defective "replicons" may be engineered and utilized. Such replicons maintain all protein encoding sequences and cis replication sequences required for RNA replication and expression within a cell, but are deleted of one or more sequences or genes required for packaging of progeny SARS virus (see for example Curtis *et al.*, J. Virol., 76:1422-1434, 2002). Figure 14 is a schematic depicting representative examples of SARS virus replicons according to the present invention. For example a SARS virus cDNA construct is generated, that is lacking one or more (or all) structural protein encoding genes, whereby the missing SARS virus gene(s) is/are replaced by the GOI, maintaining all necessary transcription signals for expression of the GOI. Operably linked with the SARS virus replicon cDNA construct is a promoter for RNA polymerase that can be used to transcribe the replicon RNA *in vivo* (*e.g.*, RNA polymerase II promoter) or in vitro (*e.g.*, bacteriophage promoter). The SARS replicon may be introduced into a susceptible cell by transfection as RNA or DNA, depending on the promoter of choice, and the transfected cells may be utilized for the evaluation of antiviral compounds. By incorporating one or more mutations rendering the replicon noncytopathic for the cells (see above), one can avoid the need for nucleic acid transfection each time an assay is to be performed.

Alternatively, SARS virus replicons may be packaged into virus like particles that allow infection of cells, rather than requiring transfection of nucleic acid molecules. A requirement for replicon packaging is that essential SARS virus gene functions deleted from the replicon (*e.g.*, one or more structural proteins) are provided in *trans* within the cell containing the replicon. A variety of methods for packaging of replicon RNA can be utilized to one of skill in the art (see for example, Curtis *et al.*, ibid: Ortego, *et al.*, J. Virol., 76:11518-11529, 2002). For example, stably transformed cell lines constitutively or inducibly expressing the required SARS virus gene functions may be utilized. Alternatively, the required SARS virus gene functions may be expressed by viral vectors that are introduced into the replicon-containing cell. Alternatively a defective interfering (DI) SARS virus derived RNA containing the required gene functions may be introduced into the replicon-containing cell. Such DI constructs used to complement missing replicon functions may be more commonly referred to as defective helper RNA or defective helpers.

Another configuration useful for cell-based antiviral screening assays according to the present invention utilizes SARS virus derived DI RNAs encoding a GOI (see for example Stirrups, *et al.*, J. Gen. Virol., 81:1687-1698, 2000; Liao, *et al.*, Virology 208:319-327, 1995).

Introduction of a SARS DI, either as cDNA linked to an RNA polymerase II promoter or as in vitro transcribed RNA, into susceptible cells also infected with SARS virus, allows for a readout of the GOI reporter product in assays.

A replicon-based system for rapid identification of coronavirus replicase inhibitors is described by Hertzig *et al.* (2004) *J Gen Virol* DOI 10.1099/vir/0/80044-0. Briefly, the system uses a non-cytopathic selectable replicon RNA that can be stably maintained in eukaryotic cells. The replicon RNA mediates reporter gene expression as a marker for coronavirus replication, and expression of the reporter can be used to test the inhibitory effect of test compounds *in vitro*, thereby allowing high throughput screening for replicase inhibitors without the need to grow infectious virus. Preferred replicon RNAs include a neomycin resistance gene in the replicase gene with a downstream reporter gene (*e.g.* GFP) that is expressed via replicase-mediated synthesis of a sub-genomic mRNA.

VI. COMPOSITIONS AND METHODS FOR TREATMENT OF SARS VIRUS INFECTION

The present invention relates to compositions and methods for the treatment and/or prevention of SARS. The invention further includes a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. Combined treatment with the lopinavir/ritonavir (Kaletra) protease inhibitor and ribavirin has shown a favorable clinical response (Chu *et al.* (2004) *Thorax* 59:252-256). In another embodiment, the antiviral compound is an inhibitor of an RNA dependent RNA polymerase. In another embodiment, a first antiviral compound that is a protease inhibitor is administered with a second antiviral compound that is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2. A combination treatment of steroids and ribavirin has been described by Fujii *et al.* (2004) *J Infect Chemother* 10:1-7. A combination treatment of corticosteroids and interferon alfacon-1 has also been reported (Loutfy *et al.* (2003) *JAMA* 290:3222-3228).

The invention further provides for a method for the treatment and/or prevention of SARS through the administration of a therapeutically effective amount of at least one antiviral

compound from among those described in the US Patents and published international patent applications listed in Table 1 and Table 2 by inhalation. In another aspect, the antiviral compound may be administered in combination with a SMIP, SMIS, or other immunomodulatory compound such as those in Table 34 and in Table 35. In one embodiment of the method, the antiviral compound is a small molecule. In another embodiment, the antiviral compound is a protease inhibitor. In a further embodiment, the antiviral protease inhibitor is a 3C-like protease inhibitor and/or a papain-like protease inhibitor. In another embodiment, the antiviral compound is an inhibitor of an RNA dependent RNA polymerase. In another embodiment, a first antiviral compound that is a protease inhibitor is administered with a second antiviral compound that is an RNA-dependent RNA polymerase inhibitor. The invention further provides for the administration of a steroidal anti-inflammatory drug in combination with at least one antiviral compound, for example, from the antiviral compounds described in the documents listed in Table 1 and Table 2. The steroidal anti-inflammatory drug may be administered by inhalation for a local effect or administered for systemic absorption such as via an oral or intravenous route.

The invention further provides for methods for treating SARS infection comprising administering a small molecule immunopotentiator (SMIP) compound either alone or in combination with an antiviral compound or in combination with a SARS vaccine. In a further embodiment, the SMIP is a compound disclosed herein or set forth in Table 34.

The invention further provides for methods for treating SARS infection comprising administering an immunosuppressant compound, optionally a small molecule suppressant (SMIS) compound either alone or in combination with an antiviral compound. In a further embodiment, the immunosuppressant compound is disclosed herein or set forth in Table 35.

The invention further provides peptidic immunomodulating compositions, that include oligo and polypeptides, capable of effecting inflammatory response in a patient. In one embodiment, the peptidic immunomodulating composition is able to stimulate human cells to produce cytokines. In another embodiment the peptidic immunomodulating composition is capable of decreasing cytokine levels in the human. Preferred Examples of peptidic immunomodulating compositions include those listed in Table 35, as well as TGFβ2, TGFβ1, TGFβ3, thymopentin (TP5), β-mercaptopropionyl-arginyl--lysyl-aspartyl-valyl-tyrosyl-cysteine amide, colostrinine, lactoferrin (LF), cyclolinopeptide A (CLA), and tuftsin (TKPR). The peptidic immunomodulating compositions of the invention may be used alone or in combination with other agents, preferably antiviral compounds, for the treatment of SARS.

The invention further provides for a kit for use by a consumer for the treatment and/or prevention of SARS. Such a kit comprises: a) a pharmaceutical composition comprising a therapeutically effective amount of at least one antiviral, SMIP, SMIS, or other immunomodulating compound from among those described in the US Patents and published international patent applications listed in Table 1, Table 2, Table 34 and Table 35 and a pharmaceutically acceptable carrier, vehicle or diluent; b) a container for holding the pharmaceutical composition; and, optionally, c) instructions describing a method of using the pharmaceutical compositions for the treatment and or the prevention of SARS. The kit may optionally contain a plurality of compounds for the treatment of SARS wherein the antiviral compounds are selected from 3C-like protease inhibitors and papain-like protease inhibitors. In a further embodiment, the kit contains an antiviral compound that is an RNA-dependent RNA polymerase inhibitor. When the kit comprises more than one antiviral, SMIP, SMIS, or other immunomodulating compound, the compounds contained in the kit may be optionally combined in the same pharmaceutical composition.

An additional aspect of the invention provides for the use of at least one of the antiviral, SMIP, SMIS, or other immunomodulating compounds described in the US Patents and published international patent applications listed in Table 1, Table 2, Table 34 and Table 35 for the manufacture of a medicament for the treatment or prevention of SARS.

An additional aspect of the invention provides for the use of at least one SMIP compound, or at least one immunosuppressant compound, or at least one SMIS compound for the manufacture of a medicament for the treatment or prevention of SARS. Preferred SMIP, immunosuppressant, and SMIS compounds are described herein.

Unless otherwise specified, the following terms, when used within Section VI: "Compositions and Methods for Treatment of SARS Virus Infection" of the present application have the meanings as defined below:

As used herein, "limit", "treat" and "treatment" are interchangeable terms as are "limiting" and "treating" and, as used herein, include preventative (*e.g.*, prophylactic) and palliative treatment or the act of providing preventative or palliative treatment. The terms include a postponement of development of SARS symptoms and/or a reduction in the severity of such symptoms that will or are expected to develop following infection with a SARS virus. The terms further include ameliorating existing SARS symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms.

Representative uses of the compositions and methods of the present invention include: the elimination or reduction of the viral load of the SARS virus in a vertebrate, including humans, the elimination or reduction of symptoms associated with SARS, and a reduction in morbidity

associated with SARS. In a SARS patient population, the use of the compositions and methods of the invention will result in the reduction in the high mortality rates associated with SARS.

Infection with the SARS virus and the symptoms associated with SARS can be treated in a subject by administering the compositions of the invention. The compositions of the invention may be administered systemically. For systemic use, the compounds herein are formulated for parenteral (*e.g.*, intravenous, subcutaneous, intramuscular, intraperitoneal, intranasal or transdermal) or enteral (*e.g.*, oral or rectal) delivery according to conventional methods. Intravenous administration can be by a series of injections or by continuous infusion over an extended period. Administration by injection or other routes of discretely spaced administration can be performed at intervals ranging from weekly to once to three times daily or more. Alternatively, the compositions disclosed herein may be administered in a cyclical manner (administration of disclosed composition, followed by no administration, followed by administration of disclosed compositions, and the like). Treatment will continue until the desired outcome is achieved.

A "subject" is a vertebrate animal including a human that is in need of treatment with the compositions, methods and kits of the present invention. The term "subject" or "subjects" is intended to refer to both the male and female gender unless one gender is specifically indicated.

"Coadministration" of a combination of a plurality of antiviral compounds means that these components can be administered together as a composition or as part of the same, unitary dosage form. "Co-administration" also includes administering a plurality of antiviral compounds separately but as part of the same therapeutic treatment program or regimen. "Co-administration" also includes administering a plurality of other agents, such as, for example an oligopeptide, a polypeptide, a peptidic immunomodulator, nucleic acid, antibodies, or a vaccine wherein the compounds or agents are administered separately but as part of the same therapeutic treatment program or regimen. The components need not necessarily be administered at essentially the same time, although they can if so desired. "Co-administration" also includes separate administration at different times and in any order. For example, where appropriate a patient may take one or more component(s) of the treatment in the morning and the one or more of the other component(s) at night.

By "antiviral compound" as used herein is meant an antiviral compound as described in the US Patents and published international patent applications listed in Table 1 and Table 2. The US Patents and published international patent applications listed in Table 1, Table 2 and Table 35 are incorporated herein in their entirety. In one embodiment, the antiviral compound is an RNA-dependent RNA polymerase. In another preferred embodiment the antiviral compound is a 3C-like protease inhibitor or a papain-like protease inhibitor. The antiviral compounds may be

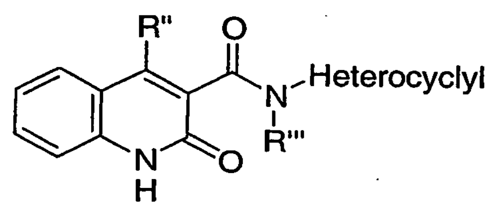
administered in the form of the acid, or of a soluble alkali metal salt or alkaline earth metal salt where appropriate.

The precise dosage of the antiviral compound will vary with the dosing schedule, the oral potency of the particular antiviral compound chosen, the age, size, sex and condition of the subject, the severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician.

Generally, an appropriate amount of antiviral compound is chosen to obtain a reduction in the load of the SARS virus in the subject and/or to obtain a reduction in the symptoms associated with SARS. For humans, an effective oral dose of antiviral compound is typically from about 1.5 to about 6000 μg/kg body weight per day and preferably about 10 to about 2000 μg/kg of body weight per day.

One of ordinary skill in the art will recognize that certain antiviral, SMIP, SMIS, and immunomodulating compounds of the invention including 3C-like protease inhibitors, papain-like protease inhibitators, and RNA-dependent RNA polymerase inhibitors will contain one or more atoms that may be in a particular stereochemical, tautomeric, or geometric configuration, giving rise to stereoisomers, tautomers and configurational isomers. All such isomers and mixtures thereof are included in this invention, when active. Crystalline and amorphous forms of the antiviral compounds of this invention are also included as are hydrates, solvates, polymorphs, and isomorphs of the antiviral compounds of the invention.

SMIP compounds of the invention include compounds are described in issued U.S. Patent Nos. 4,547,511 and 4,738,971 with the general structure (a):

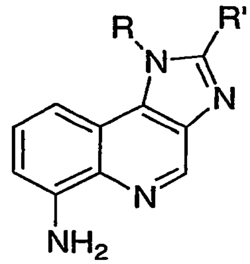


a

for the treatment of disorders responsive to agents that enhance cell-mediated immunity.

Immunostimulatory oligonucleotides and polynucleotides are described in PCT WO 98/55495 and PCT WO 98/16247. U.S. Patent Application No. 2002/0164341 describes adjuvants including an unmethylated CpG dinucleotide (CpG ODN) and a non-nucleic acid adjuvant. U.S. Patent Application No. 2002/0197269 describes compositions comprising an antigen, an antigenic CpG-ODN and a polycationic polymer.

Additionally, issued U.S. Patent Nos. 4,689,338, 5,389,640, 5,268,376, 4,929,624, 5,266,575, 5,352,784, 5,494,916, 5,482,936, 5,346,905, 5,395,937, 5,238,944, 5,525,612, WO99/29693 and U.S. Ser. No. 09/361,544 disclose compounds of the general structure (b):

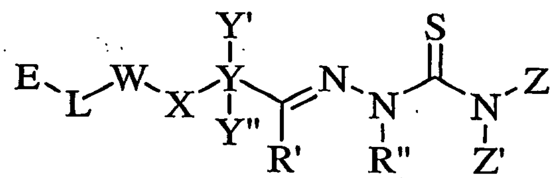


b

for the use as "immune response modifiers."

Further compounds with SMIP and antiviral activity are described below and in US Patent Application entitled Thiosemicarbazones as Anti-Virals and Immunopotentiators filed on December 29, 2003 with an attorney docket number of PP19814.004US generally disclosing compounds of the following structures:

A compound of formula c:



c

wherein: E is absent or selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

L is absent or is selected from the group consisting of oxo, amino, alkylene, substituted alkylene, alkoxy, alkylamino, aminoalkyl, heterocyclyl, carbocyclyl, and carbonyl;

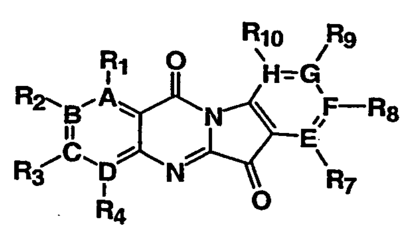
W is absent or selected from the group consisting of cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

X is absent or is selected from the group consisting of oxo, amino, alkylene, substituted alkylene, alkoxy, alkylamino, aminoalkyl, heterocyclyl, carbocyclyl, and carbonyl;

Y is selected from the group consisting of cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, and substituted heteroaryl;

Y' is absent or is selected from the group consisting of F, Cl, Br, I, nitro, alkyl, substituted alkyl, and optionally substituted heterocyclyl, amino, alkylamino, dialkylamino;
Y" is absent or is selected from the group consisting of F, Cl, Br, I, nitro, alkyl, substituted alkyl, and optionally substituted heterocyclyl, amino, alkylamino, dialkylamino;
R' is H, alkyl, or substituted alkyl;
R'' is H, or
R' and R'' are taken together to form a hetercyclic ring;
Z and Z' are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido; or
Z and Z' are taken together to form a heterocyclic group, that may be optionally substituted and the tautomers and the pharmaceutically acceptable salts, esters, or prodrugs thereof.

Further SMIP compounds are described below and in US Patent Application 10/762873, Use of Tryptanthrin Compounds for Immune Potentiation, filed on January 21, 2004 and disclosing the general embodiment of compounds represented by Formula (d):



(d)

wherein

A, B, C, D, E, F, G, and H are independently selected from carbon and nitrogen, or A and B and/or C and D can be taken together to be nitrogen or sulfur;

R_1, R_2, R_3, R_4, R_8, and R_{10} are independently selected from the group consisting of hydrogen, halogen, loweralkyl, alkyl, substituted alkyl, cycloalkyl, heterocyclyl, alkylheterocyclyl, substituted heterocyclyl, substituted alkenyl, amino, (substituted alkyl)(alkyl)amino, imino, haloloweralkyl, hydroxy, alkoxy, substituted alkoxy, hydroxyalkylthio, nitro, alkylsulfonyl, N-alkylsulfonamide, arylalkyl, arylalkylaryl, arylaryl, aryloxy, arylamino, acylamino, acyloxyamino, alkylaminoacylamino, alkylaminosulfonylamino, alkylamino, alkenylamino, dialkylamino, alkoxyalkylamino, alkoxyalkylheterocyclyl, mercaptoalkoxyalkyl, cyano, formyl, -COOR$_{11}$ wherein R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -CONR$_{12}$R$_{13}$ wherein R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues; or R_2 and R_3 taken together form a six membered aromatic ring;

R_7 and R_9 are independently selected from hydrogen, halogen, loweralkyl, haloloweralkyl, cycloalkyl, heterocyclyl, substituted heterocyclyl or heterocyclylalkyl; and

R_1, R_2, R_3, R_4, R_7, R_8, R_9,and R_{10} are absent when the ring atom to which they would otherwise be bonded is sulfur or double-bonded nitrogen; or

the a pharmaceutically acceptable salts, esters, or prodrugs thereof,

provided that R_1, R_2, R_3, R_4, R_7, R_8, R_9, and R_{10} are not all hydrogen when A, B, C, D, E, F, and H are carbon.

In one embodiment, the compounds of Formula (I) have a backbone structure wherein D is nitrogen, and A-C and E-H are carbon.

In one embodiment, when D is carbon, at least one, or at least two of R_1 - R_4, and R_7 - R_{10} are not hydrogen.

In one embodiment, R_1 through R_4, and R_8 and R_{10} are independently selected from at least two of the group consisting of hydrogen, halogen, loweralkyl, cycloalkyl, heterocyclyl, substituted heterocyclyl, alkylheterocyclyl, amino, imino, haloloweralkyl, alkoxy, nitro, alkylsulfonyl, arylalkyl, arylalkylaryl, arylaryl, aryloxy, arylamino, acylamino, acyloxyamino, alkylaminoacylamino, alkylaminosulfonylamino, alkylamino, alkenylamino, dialkylamino, alkoxyalkylamino, alkoxyalkylheterocyclyl, mercaptoalkoxyalkyl, cyano, formyl, -COOR$_{11}$ where R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -CONR$_{12}$R$_{13}$ where R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues; and R_4 is not present when D is nitrogen.

In an additional embodiment, 4A, B, C, D, E, F, G, and H are independently selected from carbon and nitrogen;

R_1, R_2, R_3, R_4, R_8 and R_{10} are independently selected from the group consisting of hydrogen, halogen, loweralkyl, alkyl, substituted alkyl, heterocyclyl, substituted heterocyclyl, substituted alkenyl, (substituted alkyl)(alkyl)amino, haloloweralkyl, hydroxy, alkoxy, substituted alkoxy, hydroxyalkylthio, nitro, N-alkylsulfonamide, cyano, -$COOR_{11}$ wherein R_{11} is hydrogen, loweralkyl, aryl, heterocyclyl, monosaccharide or disaccharide, and -$CONR_{12}R_{13}$ wherein R_{12} and R_{13} are independently selected from hydrogen, loweralkyl, aryl, heterocyclyl, saccharide, peptide and amino acid residues.

For the compounds described herein:

The term "loweralkyl" refers to branched or straight chain acyclical alkyl groups comprising one to ten carbon atoms, including, e.g., methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, neopentyl and the like.

The term "alkyl" refers to alkyl groups that do not contain heteroatoms. Thus the term includes straight chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The phrase also includes branched chain isomers of straight chain alkyl groups, including but not limited to, the following that are provided by way of example: $-CH(CH_3)_2$, $-CH(CH_3)(CH_2CH_3)$, $-CH(CH_2CH_3)_2$, $-C(CH_3)_3$, $-C(CH_2CH_3)_3$, $-CH_2CH(CH_3)_2$, $-CH_2CH(CH_3)(CH_2CH_3)$, $-CH_2CH(CH_2CH_3)_2$, $-CH_2C(CH_3)_3$, $-CH_2C(CH_2CH_3)_3$, $-CH(CH_3)CH(CH_3)(CH_2CH_3)$, $-CH_2CH_2CH(CH_3)_2$, $-CH_2CH_2CH(CH_3)(CH_2CH_3)$, $-CH_2CH_2CH(CH_2CH_3)_2$, $-CH_2CH_2C(CH_3)_3$, $-CH_2CH_2C(CH_2CH_3)_3$, $-CH(CH_3)CH_2CH(CH_3)_2$, $-CH(CH_3)CH(CH_3)CH(CH_3)_2$, $-CH(CH_2CH_3)CH(CH_3)CH(CH_3)(CH_2CH_3)$, and others. The term also includes cyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and such rings substituted with straight and branched chain alkyl groups as defined above. The term also includes polycyclic alkyl groups such as, but not limited to, adamantyl norbornyl, and bicyclo[2.2.2]octyl and such rings substituted with straight and branched chain alkyl groups as defined above. Thus, the phrase unsubstituted alkyl groups includes primary alkyl groups, secondary alkyl groups, and tertiary alkyl groups. Unsubstituted alkyl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound. Preferred unsubstituted alkyl groups include straight and branched chain alkyl

groups and cyclic alkyl groups having 1 to 20 carbon atoms. More preferred such unsubstituted alkyl groups have from 1 to 10 carbon atoms while even more preferred such groups have from 1 to 5 carbon atoms. Most preferred unsubstituted alkyl groups include straight and branched chain alkyl groups having from 1 to 3 carbon atoms and include methyl, ethyl, propyl, and $-CH(CH_3)_2$.

The phrase "substituted alkyl" refers to an unsubstituted alkyl group as defined above in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen and non-carbon atoms such as, but not limited to, a halogen atom in halides such as F, Cl, Br, and I; a phosphorus atom in groups such as phosphate and dialkyl alkylphosphonate; oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. Substituted alkyl groups also include groups in which one or more bonds to a carbon(s) or hydrogen(s) atom is replaced by a bond to a heteroatom such as oxygen in carbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles. Preferred substituted alkyl groups include, among others, alkyl groups in which one or more bonds to a carbon or hydrogen atom is/are replaced by one or more bonds to fluorine atoms. One example of a substituted alkyl group is the trifluoromethyl group and other alkyl groups that contain the trifluoromethyl group. Other alkyl groups include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a hydroxyl, alkoxy, aryloxy group, or heterocyclyloxy group. Still other alkyl groups include alkyl groups that have an amine, alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine, diarylamine, heterocyclylamine, (alkyl)(heterocyclyl)amine, (aryl)(heterocyclyl)amine, or diheterocyclylamine group.

The term "alkoxy" refers to RO- wherein R, for example, is alkyl such as loweralkyl defined above. Representative examples of loweralkyl alkoxy groups include methoxy, ethoxy, t-butoxy and the like.

The phrase "substituted alkoxy" refers to RO-, where R is, for example, an alkyl substituted, for example, with a halogen. RO is for example OCF_3.

The term "alkenyl" refers to a branched or straight chain groups comprising two to twenty carbon atoms that also comprises one or more carbon-carbon double bonds. Representative alkenyl groups include prenyl, 2-propenyl (i.e., allyl), 3-methyl-2-butenyl, 3,7-dimethyl-2,6-octadienyl, 4,8-dimethyl-3,7-nonadienyl, 3,7,11-trimethyl-2,6,10-dodecatrienyl and the like.

The phrase "substituted alkenyl" refers to alkenyl groups that are substituted, for example, diethyl hex-5-enylphosponate, and others with an alkyl or substituted alkyl group such as dialkyl phosphate or an ester such as an acetate ester.

The phrase "dialkyl amino" refers to an amino group substituted with two alkyl groups such as C1-20 alkyl groups.

The phrase "substituted dialkyl amino" refers to a dialkylamino substituted, for example, with a carboxylic acid, ester, hydroxy or alkoxy.

The term "hydroxyalkylthio" refers to a thio radical to which is appended a hydroxyalkyl group, where the alkyl is for example lower alkyl. An example is hydroxyethylthio, -SCH_2CH_2OH.

The term "N-alkylsulfonamide" refers to the group -SO_2NHalkyl, where alkyl is, for example, octyl.

The term "alkynyl" refers to a branched or straight chain comprising two to twenty carbon atoms that also comprises one or more carbon-carbon triple bonds. Representative alkynyl groups include ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

The term "aryl" refers to aryl groups that do not contain heteroatoms. Thus the term includes, but is not limited to, groups such as phenyl, biphenyl, anthracenyl, naphthenyl by way of example. Although the phrase "unsubstituted aryl" includes groups containing condensed rings such as naphthalene, it does not include aryl groups that have other groups such as alkyl or halo groups bonded to one of the ring members, as aryl groups such as tolyl are considered herein to be substituted aryl groups as described below. A preferred unsubstituted aryl group is phenyl. Unsubstituted aryl groups may be bonded to one or more carbon atom(s), oxygen atom(s), nitrogen atom(s), and/or sulfur atom(s) in the parent compound, however.

The phrase "substituted aryl group" has the same meaning with respect to aryl groups that substituted alkyl groups had with respect to alkyl groups. However, a substituted aryl group also includes aryl groups in which one of the aromatic carbons is bonded to one of the non-carbon or non-hydrogen atoms described above and also includes aryl groups in which one or more aromatic carbons of the aryl group is bonded to a substituted and/or unsubstituted alkyl, alkenyl,

or alkynyl group as defined herein. This includes bonding arrangements in which two carbon atoms of an aryl group are bonded to two atoms of an alkyl, alkenyl, or alkynyl group to define a fused ring system (e.g. dihydronaphthyl or tetrahydronaphthyl). Thus, the phrase "substituted aryl" includes, but is not limited to tolyl, and hydroxyphenyl among others.

The term "arylalkyl" refers to a loweralkyl radical to which is appended an aryl group. Representative arylalkyl groups include benzyl, phenylethyl, hydroxybenzyl, fluorobenzyl, fluorophenylethyl and the like.

The phrase "unfused arylaryl" refers to a group or substituent to which two aryl groups, that are not condensed to each other, are bound. Exemplary unfused arylaryl compounds include, for example, phenylbenzene, diphenyldiazene, 4-methylthio-1-phenylbenzene, phenoxybenzene, (2-phenylethynyl)benzene, diphenyl ketone, (4-phenylbuta-1,3-diynyl)benzene, phenylbenzylamine, (phenylmethoxy)benzene, and the like. Preferred substituted unfused arylaryl groups include: 2-(phenylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 1,4-diphenylbenzene, N-[4-(2-phenylethynyl)phenyl]-2-[benzylamino]acetamide, 2-amino-N-[4-(2-phenylethynyl)phenyl]propanamide, 2-amino-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(cyclopropylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(ethylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-[(2-methylpropyl)amino]-N-[4-(2-phenylethynyl)phenyl]acetamide, 5-phenyl-2H-benzo[d]1,3-dioxolene, 2-chloro-1-methoxy-4-phenylbenzene, 2-[(imidazolylmethyl)amino]-N-[4-(2-phenylethynyl)phenyl]acetamide, 4-phenyl-1-phenoxybenzene, N-(2-aminoethyl)[4-(2-phenylethynyl)phenyl]carboxamide, 2-{[(4-fluorophenyl)methyl]amino}-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-{[(4-methylphenyl)methyl]amino}-N-[4-(2-phenylethynyl)phenyl]acetamide, 4-phenyl-1-(trifluoromethyl)benzene, 1-butyl-4-phenylbenzene, 2-(cyclohexylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(ethylmethylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, 2-(butylamino)-N-[4-(2-phenylethynyl)phenyl]acetamide, N-[4-(2-phenylethynyl)phenyl]-2-(4-pyridylamino)acetamide, N-[4-(2-phenylethynyl)phenyl]-2-(quinuclidin-3-ylamino)acetamide, N-[4-(2-phenylethynyl)phenyl]pyrrolidin-2-ylcarboxamide, 2-amino-3-methyl-N-[4-(2-phenylethynyl)phenyl]butanamide, 4-(4-phenylbuta-1,3-diynyl)phenylamine, 2-(dimethylamino)-N-[4-(4-phenylbuta-1,3-diynyl)phenyl]acetamide, 2-(ethylamino)-N-[4-(4-phenylbuta-1,3-diynyl)phenyl]acetamide, 4-ethyl-1-phenylbenzene, 1-[4-(2-phenylethynyl)phenyl]ethan-1-one, N-(1-carbamoyl-2-hydroxypropyl)[4-(4-phenylbuta-1,3-diynyl)phenyl]carboxamide, N-[4-(2-phenylethynyl)phenyl]propanamide, 4-methoxyphenyl phenyl ketone, phenyl-N-benzamide, (tert-butoxy)-N-[(4-phenylphenyl)methyl]carboxamide, 2-

(3-phenylphenoxy)ethanehydroxamic acid, 3-phenylphenyl propanoate, 1-(4-ethoxyphenyl)-4-methoxybenzene, and [4-(2-phenylethynyl)phenyl]pyrrole.

The phrase "unfused heteroarylaryl" refers to a unfused arylaryl group where one of the aryl groups is a heteroaryl group. Exemplary heteroarylaryl groups include, for example, 2-phenylpyridine, phenylpyrrole, 3-(2-phenylethynyl)pyridine, phenylpyrazole, 5-(2-phenylethynyl)-1,3-dihydropyrimidine-2,4-dione, 4-phenyl-1,2,3-thiadiazole, 2-(2-phenylethynyl)pyrazine, 2-phenylthiophene, phenylimidazole, 3-(2-piperazinylphenyl)furan, 3-(2,4-dichlorophenyl)-4-methylpyrrole, and the like. Preferred substituted unfused heteroarylaryl groups include: 5-(2-phenylethynyl)pyrimidine-2-ylamine, 1-methoxy-4-(2-thienyl)benzene, 1-methoxy-3-(2-thienyl)benzene, 5-methyl-2-phenylpyridine, 5-methyl-3-phenylisoxazole, 2-[3-(trifluoromethyl)phenyl]furan, 3-fluoro-5-(2-furyl)-2-methoxy-1-prop-2-enylbenzene, (hydroxyimino)(5-phenyl(2-thienyl))methane, 5-[(4-methylpiperazinyl)methyl]-2-phenylthiophene, 2-(4-ethylphenyl)thiophene, 4-methylthio-1-(2-thienyl)benzene, 2-(3-nitrophenyl)thiophene, (tert-butoxy)-N-[(5-phenyl(3-pyridyl))methyl]carboxamide, hydroxy-N-[(5-phenyl(3-pyridyl))methyl]amide, 2-(phenylmethylthio)pyridine, and benzylimidazole.

The phrase "unfused heteroarylheteroaryl" refers to an unfused arylaryl group where both of the aryl groups is a heteroaryl group. Exemplary heteroarylheteroaryl groups include, for example, 3-pyridylimidazole, 2-imidazolylpyrazine, and the like. Preferred substituted unfused heteroarylheteroaryl groups include: 2-(4-piperazinyl-3-pyridyl)furan, diethyl(3-pyrazin-2-yl(4-pyridyl))amine, and dimethyl{2-[2-(5-methylpyrazin-2-yl)ethynyl](4-pyridyl)}amine.

The phrase "fused arylaryl" refers to an aryl group as previously defined that is condensed, and fully conjugated to an aryl group. Representative fused arylaryl groups include biphenyl, 4-(1-naphthyl)phenyl, 4-(2-naphthyl)phenyl and the like.

The phrase "fused heteroarylaryl" refers to an aryl group as previously defined that is condensed, and fully conjugated with a heteroaryl group. Representative fused heteroarylaryl groups include quinoline, quinazoline and the like.

The phrase "fused heteroarylheteroaryl" refers to a heteroaryl group as previously defined that is condensed, and fully conjugated with another heteroaryl group. Representative fused heteroarylheteroaryl groups include pyrazalopyrimidine, imidazoquinoline and the like.

The term "aryloxy" refers to RO- wherein R is an aryl group. Representative arylalkoxy group include benzyloxy, phenylethoxy and the like.

The term "arylalkoxy" refers to a lower alkoxy radical to which is appended an aryl group. Representative arylalkoxy group include benzyloxy, phenylethoxy and the like.

The term "aryloxyaryl" refers to an aryl radical to which is appended an aryloxy group. Representative aryloxyaryl groups include 4-phenoxyphenyl, 3-phenoxyphenyl, 4-phenoxy-1-naphthyl, 3-phenoxy-1-naphthyl and the like.

The term "aryloxyarylalkyl" refers to an arylalkyl radical to which is appended an aryloxy group. Representative aryloxyarylalkyl groups include 4-phenoxyphenylmethyl, 3-phenoxyphenylmethyl, 4-phenoxyphenylethyl, 3-phenoxy-phenylethyl and the like.

The term "arylalkoxyaryl" refers to an aryl radical to which is appended an arylalkoxy group. Representative arylalkoxyaryl groups include 4-benzyloxylphenyl, 3-benzyloxyphenyl and the like.

The term "arylalkoxyarylalkyl" refers to an arylalkyl radical to which is appended an arylalkoxy group. Representative arylalkoxyarylalkyl groups include 4-benzyloxylbenzyl, 3-benzyloxybenzyl and the like.

The term "cycloalkyl" refers to an alicyclic group comprising from 3 to 7 carbon atoms including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cycloalkylalkyl" refers to a loweralkyl radical to which is appended a cycloalkyl group. Representative examples of cycloalkylalkyl include cyclopropylmethyl, cyclohexylmethyl, 2-(cyclopropyl)ethyl and the like.

The term "halogen" refers to iodine, bromine, chlorine or fluorine; "halo" refers to iodo, bromo, chloro or fluoro.

The term "haloalkyl" refers to a lower alkyl radical, as defined above, bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term "heterocyclyl" (or heterocyclic, or heterocyclo) refers to both aromatic and nonaromatic ring compounds including monocyclic, bicyclic, and polycyclic ring compounds such as, but not limited to, quinuclidyl, containing 3 or more ring members of which one or more is a heteroatom such as, but not limited to, N, O, and S. Although the phrase "unsubstituted heterocyclyl" includes condensed heterocyclic rings such as benzimidazolyl, it does not include heterocyclyl groups that have other groups such as alkyl or halo groups bonded to one of the ring members as compounds such as 2-methylbenzimidazolyl are substituted heterocyclyl groups. Examples of heterocyclyl groups include, but are not limited to: unsaturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but not limited to pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl etc.), tetrazolyl, (e.g. 1H-tetrazolyl, 2H tetrazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 4 nitrogen atoms such as, but

not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl; condensed unsaturated heterocyclic groups containing 1 to 4 nitrogen atoms such as, but not limited to, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl; unsaturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, oxazolyl, isoxazolyl, oxadiazolyl (e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms such as, but not limited to, morpholinyl; unsaturated condensed heterocyclic groups containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, benzoxazolyl, benzoxadiazolyl, benzoxazinyl (e.g. 2H-1,4-benzoxazinyl etc.); unsaturated 3 to 8 membered rings containing 1 to 3 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolyl, isothiazolyl, thiadiazolyl (e.g. 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.); saturated 3 to 8 membered rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, thiazolodinyl; saturated and unsaturated 3 to 8 membered rings containing 1 to 2 sulfur atoms such as, but not limited to, thienyl, dihydrodithiinyl, dihydrodithionyl, tetrahydrothiophene, tetrahydrothiopyran; unsaturated condensed heterocyclic rings containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms such as, but not limited to, benzothiazolyl, benzothiadiazolyl, benzothiazinyl (e.g. 2H-1,4-benzothiazinyl, etc.), dihydrobenzothiazinyl (e.g. 2H-3,4-dihydrobenzothiazinyl, etc.), unsaturated 3 to 8 membered rings containing oxygen atoms such as, but not limited to furyl; unsaturated condensed heterocyclic rings containing 1 to 2 oxygen atoms such as benzodioxolyl (e.g. 1,3-benzodioxoyl, etc.); unsaturated 3 to 8 membered rings containing an oxygen atom and 1 to 2 sulfur atoms such as, but not limited to, dihydrooxathiinyl; saturated 3 to 8 membered rings containing 1 to 2 oxygen atoms and 1 to 2 sulfur atoms such as 1,4-oxathiane; unsaturated condensed rings containing 1 to 2 sulfur atoms such as benzothienyl, benzodithiinyl; and unsaturated condensed heterocyclic rings containing an oxygen atom and 1 to 2 oxygen atoms such as benzoxathiinyl. Heterocyclyl group also include those described above in which one or more S atoms in the ring is double-bonded to one or two oxygen atoms (sulfoxides and sulfones). For example, heterocyclyl groups include tetrahydrothiophene, tetrahydrothiophene oxide, and tetrahydrothiophene 1,1-dioxide. Preferred heterocyclyl groups contain 5 or 6 ring members. More preferred heterocyclyl groups include morpholine, piperazine, piperidine, pyrrolidine, imidazole, pyrazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, thiomorpholine, thiomorpholine in which the S atom of the thiomorpholine is bonded to one or

more O atoms, pyrrole, homopiperazine, oxazolidin-2-one, pyrrolidin-2-one, oxazole, quinuclidine, thiazole, isoxazole, furan, and tetrahydrofuran.

The phrase "substituted heterocyclyl" refers to an heterocyclyl group as defined above in which one of the ring members is bonded to a non-hydrogen atom such as described above with respect to substituted alkyl groups and substituted aryl groups. Examples, include, but are not limited to, 2-methylbenzimidazolyl, 5-methylbenzimidazolyl, 5-chlorobenzthiazolyl, 1-methyl piperazinyl, and 2-chloropyridyl among others.

"Aminosulfonyl" refers to the group $-S(O)_2-NH_2$. "Substituted aminosulfonyl" refersto the group $-S(O)_2$-NRR' where R is loweralkyl and R' is hydrogen or a loweralkyl. The term "aralkylaminosulfonlyaryl" refers to the group $-$aryl-$S(O)_2$–NH-aralkyl, where the aralkyl is loweraralkyl.

"Carbonyl" refers to the divalent group –C(O)-.

"Carbonyloxy" refers generally to the group –C(O)-O-,. Such groups include esters, -C(O)-O-R, where R is loweralkyl, cycloalkyl, aryl, or loweraralkyl. The term "carbonyloxycycloalkyl" refers generally to both an "carbonyloxycarbocycloalkyl" and an "carbonyloxyheterocycloalkyl", i.e., where R is a carbocycloalkyl or heterocycloalkyl, respectively. The term "arylcarbonyloxy" refers to the group –C(O)-O-aryl, where aryl is a mono- or polycyclic, carbocycloaryl or heterocycloaryl. The term "aralkylcarbonyloxy" refers to the group –C(O)-O-aralkyl, where the aralkyl is loweraralkyl.

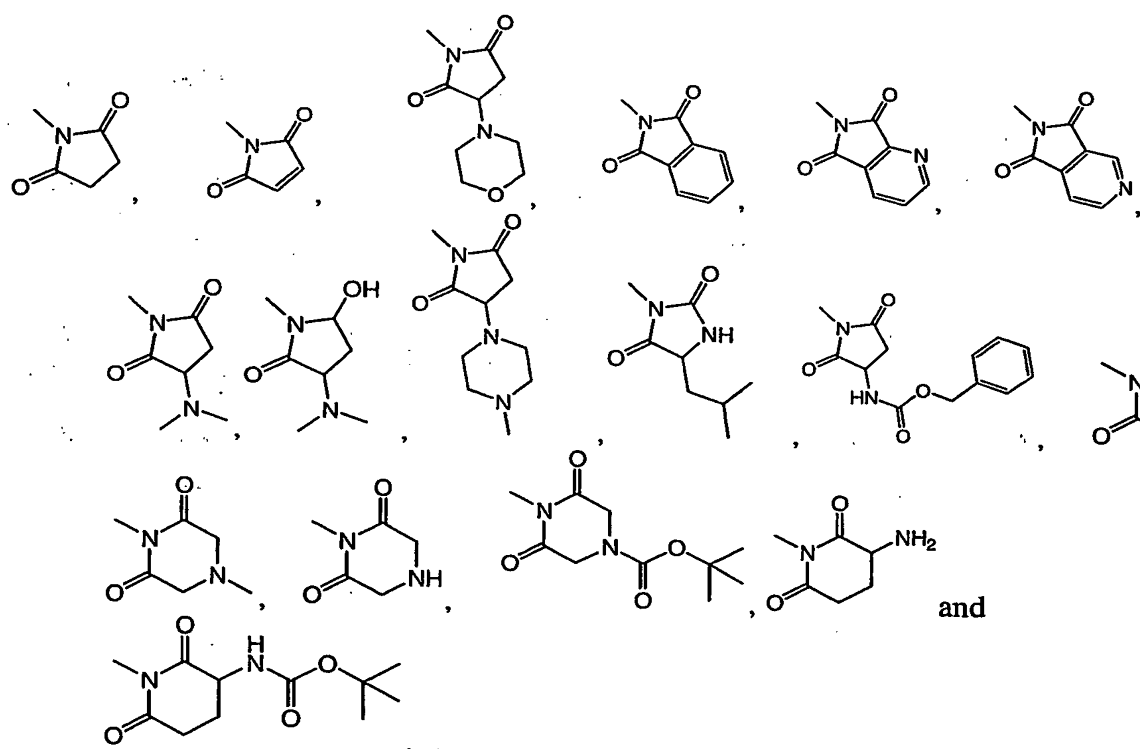
The term "sulfonyl" refers to the group $-SO_2-$. "Alkylsulfonyl" refers to a substituted sulfonyl of the structure $-SO_2R$ - in which R is alkyl. Alkylsulfonyl groups employed in compounds of the present invention are typically loweralkylsulfonyl groups having from 1 to 6 carbon atoms in its backbone structure. Thus, typical alkylsulfonyl groups employed in compounds of the present invention include, for example, methylsulfonyl (i.e., where R is methyl), ethylsulfonyl (i.e., where R is ethyl), propylsulfonyl (i.e., where R is propyl), and the like. The term "arylsulfonyl" refersto the group $-SO_2$-aryl. The term "aralkylsulfonyl" refers to the group $-SO_2$-aralkyl, in which the aralkyl is loweraralkyl. The term "sulfonamido" refers to $-SO_2NH2$.

The term "carbonylamino" refers to the divalent group -NH-C(O)- in which the hydrogen atom of the amide nitrogen of the carbonylamino group can be replaced a loweralkyl, aryl, or loweraralkyl group. Such groups include moieties such as carbamate esters (-NH-C(O)-O-R) and amides –NH-C(O)-O-R, where R is a straight or branched chain loweralkyl, cycloalkyl, or aryl or loweraralkyl. The term "loweralkylcarbonylamino" refers to alkylcarbonylamino where R is a

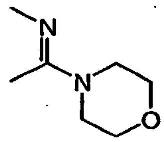
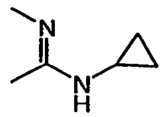
loweralkyl having from 1 to about 6 carbon atoms in its backbone structure. The term "arylcarbonylamino" refers to group –NH-C(O)-R where R is an aryl. Similarly, the term "aralkylcarbonylamino " refers to carbonylamino where R is a lower aralkyl.

The term "guanidino" or "guanidyl" refers to moieties derived from guanidine, H2N-C(=NH)-NH_2. Such moieties include those bonded at the nitrogen atom carrying the formal double bond (the "2"-position of the guanidine, e.g., diaminomethyleneamino, $(H2N)_2C$=NH-) and those bonded at either of the nitrogen atoms carrying a formal single bond (the "1-" and/or "3"-positions of the guandine, e.g., H_2N-C(=NH)-NH-). The hydrogen atoms at any of the nitrogens can be replaced with a suitable substituent, such as loweralkyl, aryl, or loweraralkyl.

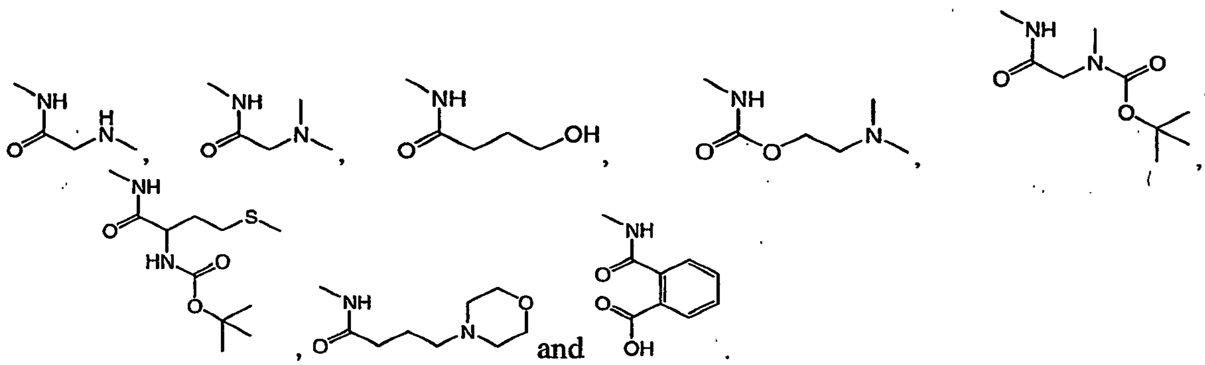
Representative cycloimido and heterocycloimido groups include, for example, those shown below. These cycloimido and heterocycloimido can be further substituted and may be attached at various positions as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



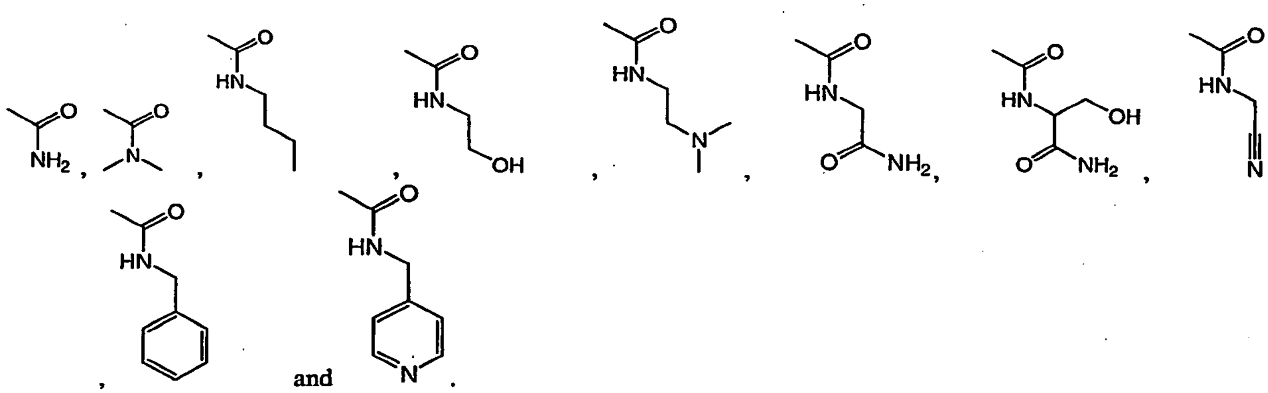
Representative substituted amidino and heterocycloamidino groups include, for example, those shown below. These amidino and heterocycloamidino groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.

 and .

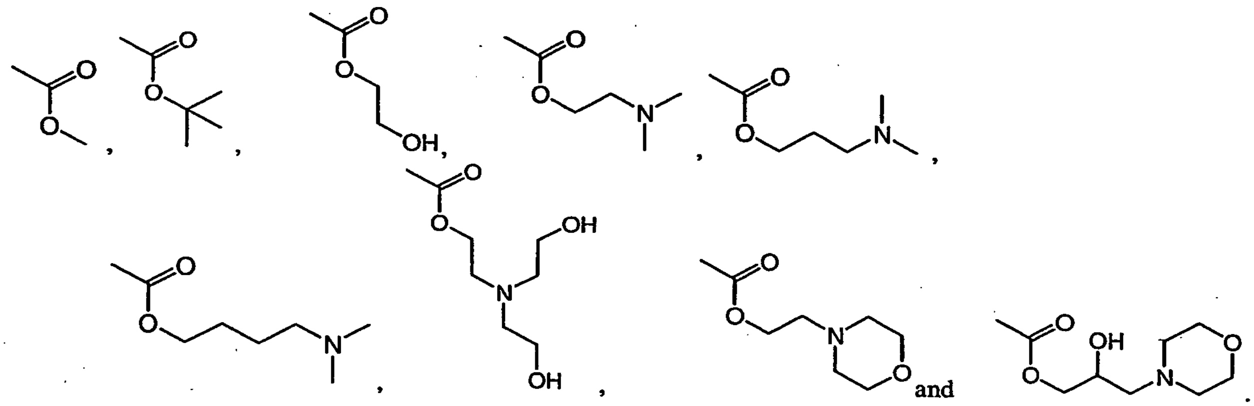
Representative substituted alkylcarbonylamino, alkyloxycarbonylamino, aminoalkyloxycarbonylamino, and arylcarbonylamino groups include, for example, those shown below. These groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



Representative substituted aminocarbonyl groups include, for example, those shown below. These can heterocyclo groups be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



Representative substituted alkoxycarbonyl groups include, for example, those shown below. These alkoxycarbonyl groups can be further substituted as will be apparent to those having skill in the organic and medicinal chemistry arts in conjunction with the disclosure herein.



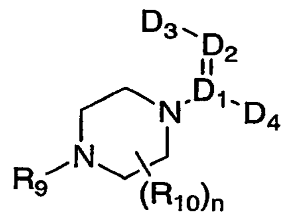
"Substituted" refers to the definite replacement of hydrogen with one or more monovalent or divalent radicals. Suitable substitution groups include, those described herein for particular groups, as well as hydroxyl, nitro, amino, imino, cyano, halo, thio, thioamido, amidino, imidino, oxo, oxamidino, methoxamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, alkyl, substituted alkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxy-alkyl, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylthio, aminoalkyl, cyanoalkyl, benzyl, pyridyl, pyrazolyl, pyrrole, thiophene, imidazolyl, and the like.

The term "linking moiety" refers to a covalent bond or an uncyclized divalent group, such as, for example, -CO-, -O-, -S-, $-CH_2-$, -NH-, and substituted or unsubstituted alkyl, alkenyl, alkynyl, carbonyl, alkoxycarbonyl groups as defined herein.

The term "SMIP compound" refers to small molecule immunopotentiating compounds, that include small molecule compounds below about MW 1000 g/mol, preferably MW 800 g/mol that are capable of stimulating or modulating a pro-inflammatory response in a patient. In an embodiment, the SMIP compounds are able to stimulate human peripheral blood mononuclear cells to produce cytokines. Preferred SMIP compounds and derivatives thereof include, for example, aminoazavinyl compounds, benzazole compounds, acylpiperazine compounds, indoledione compounds, tetrahydroisoquinoline (THIQ) compounds, anthraquinone compounds, indanedione compounds, pthalimide compounds, benzocyclodione compounds, aminobenzimidazole quinolinone (ABIQ) compounds, hydraphthalimide compounds, pyrazolopyrimidine compounds, quinazilinone compounds, quinoxaline compounds, triazine compounds, tetrahydropyrrolidinoquinoxaline compounds, pyrrole compounds, benzophenone compounds, sterol compound, and isoxazole compounds.

The term "SMIS compound" refers to small molecule immunosuppressant compounds, that include small molecule compounds below about about MW 1000 g/mol, preferably MW 800 g/mol, capable of suppressing or modulating a pro-inflammatory response in a patient.

Acylpiperazine compounds as described throughout this application include compounds of formula (III) as shown below:



III

wherein,

R_9 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, arylalkenyl, heteroarylalkyl, and heteroarylalkenyl;

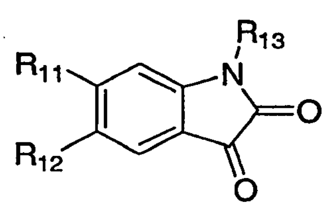
R_{10} is substituted or unsubstituted alkyl;

n is an integer from 0-2; and

if D_1 is carbon than D_2 is oxygen, D_3 is absent, and D_4 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, carbocycyl, alkoxyaryl, fused arylaryl, fused arylheteroaryl, and fused heteroarylaryl; or,

if D_1 is nitrogen than D_2 is nitrogen, D_4 is absent, and D_3 is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, carbocycyl, alkoxyaryl, fused arylaryl, fused arylheteroaryl, and fused heteroarylaryl.

Indoledione compounds as described throughout this application include compounds of formula (IV) as shown below:



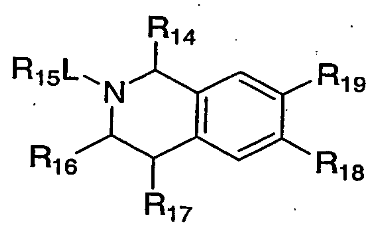
IV

wherein,

R_{11} and R_{12} are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups; and,

R_{13} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, and alkylbenzyl.

Tetrahydroisoquinoline (THIQ) compounds as described throughout this application include compounds of formula (V) as shown below:



V

wherein,

L is a covalent bond or selected from the group consisting of $-CH_2-$, -CO-, -O-, -S-, CHF, -NH-, $-NR_{20}-$, where R_{20} is lower alkyl;

R_{14} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl;

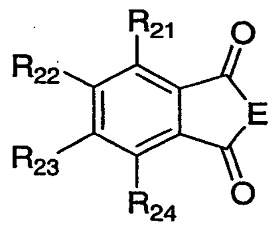
R_{15} is selected from the group consisting of substituted or unsubstituted carbocyclyl, aryl, arylalkyl, alkoxyaryl, heteroaryl, heterocyclyl;

R_{16} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl;

R_{17} is selected from the group consisting of hydrogen, halogen, and substituted or unsubstituted alkyl; and,

R_{18} and R_{19} are independently selected from the group consisting of H, hydroxy, halogen, alkoxy, amino, unsubstituted alkyl, substituted alkyl, and alkylamino.

Benzocyclodione compounds as described throughout this application include compounds of formula (VI) as shown below:



VI

wherein,

E is selected from the group consisting of NR_{25} or $CR_{26}R_{27}$;

R_{21}, R_{23}, and R_{24} are independently selected from the group consisting of H, hydroxy, halogen, alkoxy, amino, unsubstituted alkyl, substituted alkyl, and alkylamino;

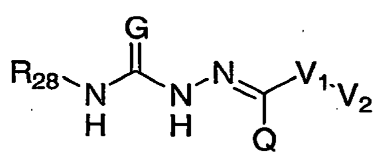
R_{22} is selected from the group consisting or H, hydroxy, halogen, alkoxy, amino, and unsubstituted or substituted alkyl, and alkylamino, arylalkyl, heteroarylalkyl, aryl, heteroaryl, arylcarbonyl, heterocyclyl, heterocyclylalkyl, and heteroarylcarbonyl;

R_{25} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, heterocyclyl, carbocyclyl, arylalkyl, heteroarylalkyl, and heterocyclyalkyl;

R_{26} is selected from the group consisting of H, halogen, hydroxy, amino, and substituted or unsubstituted alkyl, carbonylalkyl, and alkylcarbonylalkyl; and,

R_{27} is selected from the group aryl, arylalkyl, heteroarylalkyl, heterocyclyl, heterocyclylalkyl, carbocyclyl, arylcarbonylalkyl, and arylalkylcarbonyl.

Aminoazavinyl compounds as described throughout this application include compounds of formula (VII) as shown below:



VII

wherein,

G is either S or NH;

R_{28} is selected from the group consisting of H, and substituted or unsubstituted alkyl, aryl, heteroaryl, heteroarylalkyl, arylalkyl, carbocyclyl, carbocyclylalkyl, heterocyclyl, and heterocyclylalkyl;

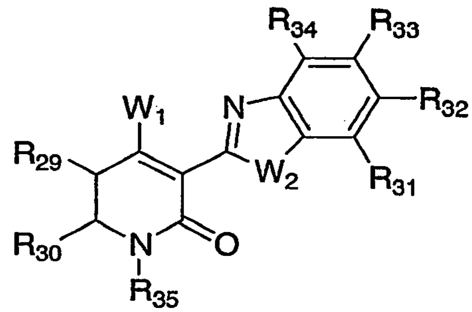
Q is selected from the group consisting of hydrogen, substituted alkyl, unsubstituted alkyl, and aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted

heterocyclyl, fused or unfused arylaryl, substituted arylaryl, arylheteroaryl, substituted arylheteroaryl, heteroarylheteroaryl, and substituted heteroarylheteroaryl;

V_1 is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido; and,

V_2 is selected from the group consisting of hydrodgen, halogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, alkoxy, substituted alkoxy, aminocarbonyl, alkoxycarbonyl, carboxyl sulfonyl, methanesulfonyl, and substituted or unsubstituted alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, cycloamidino, cycloalkyl, cycloimido, arylsulfonyl and arylsulfonamido.

Lactam compounds as described throughout this application include compounds of formula (VIII) as shown below:



VIII

wherein,

W_1 is selected from the group consisting of –OH, -OR_{36} groups, -$NR_{37}R_{38}$;

W2 is selected from the group consisting of O, S, and NR_{39} groups;

R_{29} and R_{30} join to form a 5 to 6 membered substituted or unsubstituted ring comprising all carbon atoms or at least one O, N, or S atom;

R_{35} and R_{39} may be the same or different and are selected from the group consisting of H, -OH substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, -C(=O)H, -C(=O)-alkyl groups, and –C(=O)-aryl groups;

R_{31}, R_{32}, R_{33}, and R_{34} may be the same or different and are independently selected from the group consisting of H, Cl, Br, F, I, -NO_2, -CN, -OH, -OR_{40} groups, -$NR_{41}R_{42}$ groups, -C(=O)R_{43} groups, -SH groups, substituted and unsubstituted amidinyl groups, substituted and unsubstituted guanidinyl groups, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted alkenyl groups, substituted and unsubstituted alkynyl groups, substituted and unsubstituted heterocyclyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted heterocyclylalkyl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (alkyl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted (aryl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups;

R_{36} is selected from the group consisting of substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl

groups, substituted and unsubstituted heterocyclylalkyl groups, -C(=O)H, -C(=O)-alkyl groups, –C(=O)-aryl groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, -C(=O)NH_2, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -C(=O)N$(alkyl)_2$ groups, -C(=O)N$(aryl)_2$ groups, -C(=O)N(alkyl)(aryl) groups, -NH_2, -NH(alkyl) groups, -NH(aryl) groups, -N$(alkyl)_2$ groups, -N(alkyl)(aryl) groups, -N$(aryl)_2$ groups, -C(=O)NH(heterocyclyl) groups, -C(=O)N$(heterocyclyl)_2$ groups, -C(=O)N(alkyl)(heterocyclyl) groups, and -C(=O)N(aryl)(heterocyclyl) groups;

R_{37} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, and substituted and unsubstituted heterocyclyl groups;

R_{38} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl groups, -OH, alkoxy groups, aryloxy groups, -NH_2, substituted and unsubstituted heterocyclylalkyl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted alkylamino groups, substituted and unsubstituted arylamino groups, substituted and unsubstituted dialkylamino groups, substituted and unsubstituted diarylamino groups, substituted and unsubstituted (alkyl)(aryl)amino groups, -C(=O)H, -C(=O)-alkyl groups, -C(=O)-aryl groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, -C(=O)NH_2, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -C(=O)N$(alkyl)_2$ groups, -C(=O)N$(aryl)_2$ groups, -C(=O)N(alkyl)(aryl) groups, -C(=O)-heterocyclyl groups, -C(=O)-O-heterocyclyl groups, -C(=O)NH(heterocyclyl) groups, -C(=O)-N$(heterocyclyl)_2$ groups, -C(=O)-N(alkyl)(heterocyclyl) groups, -C(=O)-N(aryl)(heterocyclyl) groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (alkyl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted (aryl)(heterocyclyl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups;

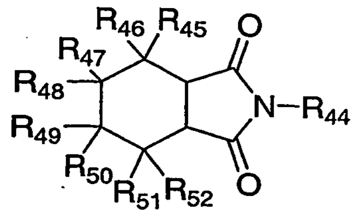
R_{41} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, and substituted and unsubstituted heterocyclyl groups;

R_{42} is selected from the group consisting of H, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, substituted and unsubstituted heterocyclyl groups, -C(=O)H, -C(=O)-alkyl groups, –C(=O)-aryl groups, -C(=O)NH_2, -C(=O)NH(alkyl) groups, -C(=O)NH(aryl) groups, -C(=O)N$(alkyl)_2$ groups, -C(=O)N$(aryl)_2$ groups, -C(=O)N(alkyl)(aryl) groups, -C(=O)O-alkyl groups, -C(=O)O-aryl groups, substituted and unsubstituted aminoalkyl groups, substituted and unsubstituted alkylaminoalkyl groups, substituted and unsubstituted dialkylaminoalkyl groups, substituted and unsubstituted arylaminoalkyl groups, substituted and unsubstituted diarylaminoalkyl groups, substituted and unsubstituted (alkyl)(aryl)aminoalkyl groups, substituted and unsubstituted heterocyclylalkyl groups, -C(=O)-heterocyclyl groups, -C(=O)-O-heterocyclyl groups, -C(=O)NH(heterocyclyl) groups, -C(=O)-N$(heterocyclyl)_2$ groups, -C(=O)-N(alkyl)(heterocyclyl) groups, -C(=O)-N(aryl)(heterocyclyl) groups, substituted and unsubstituted heterocyclylaminoalkyl groups, substituted and unsubstituted diheterocyclylaminoalkyl groups, substituted and unsubstituted (heterocyclyl)(alkyl)aminoalkyl groups, substituted and unsubstituted (heterocyclyl)(aryl)aminoalkyl groups, substituted and unsubstituted hydroxyalkyl groups, substituted and unsubstituted alkoxyalkyl groups, substituted and unsubstituted aryloxyalkyl groups, and substituted and unsubstituted heterocyclyloxyalkyl groups; and

R_{43} is selected from the group consisting of H, -NH_2, -NH(alkyl) groups, -NH(aryl) groups, -N$(alkyl)_2$ groups, -N$(aryl)_2$ groups, -N(alkyl)(aryl) groups, -NH(heterocyclyl) groups, -N(heterocyclyl)(alkyl) groups, -N(heterocyclyl)(aryl) groups, -N$(heterocyclyl)_2$ groups, substituted and unsubstituted alkyl groups, substituted and unsubstituted aryl groups, -OH, substituted and unsubstituted alkoxy groups, substituted and unsubstituted heterocyclyl groups, substituted and unsubstituted aryloxy groups, heterocyclyloxy groups, -NHOH, -N(alkyl)OH groups, -N(aryl)OH groups, -N(alkyl)O-alkyl groups, -N(aryl)O-alkyl groups, -N(alkyl)O-aryl groups, and -N(aryl)O-aryl groups.

Preferably R_{29} and R_{30} join together to form a substituted or unsubstituted phenyl ring.

Hydropthalamide compounds as described throughout this application include compounds of formula (IX) as shown below:



IX

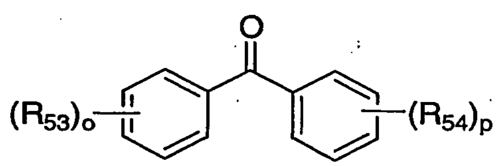
wherein,

R_{44} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, fused arylaryl, unfused arylaryl, fused heteroarylaryl, unfused heteroarylaryl, fused arylheteroaryl, and unfused arylheteroaryl;

R_{45}, R_{47}, R_{49}, and R_{51} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl; and

R_{46}, R_{48}, R_{50}, and R_{52} may be the same or different and are independently selected from the group consisting of H, halogen, and substituted or unsubstituted alkyl groups.

Benzophenone compounds as described throughout this application include compounds of formula (X) as shown below:



X

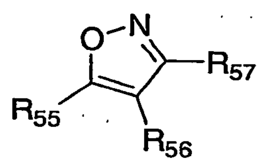
wherein,

R_{53} is independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl;

R_{54} is independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl; and

o and p are integers from 0-4.

Isoxazole compounds as described throughout this application include compounds of formula (XI) as shown below:



XI

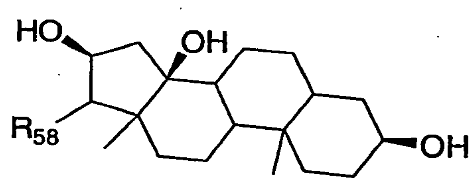
wherein,

R_{55} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl;

R_{56} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl; and,

R_{57} is selected from the group consisting of H, halogen, hydoxy, and substituted or unsubstituted alkyl, aryl, heteroaryl, heterocyclyl, and carbonyl.

Sterol compounds as described throughout this application include compounds of formula (XII) as shown below:



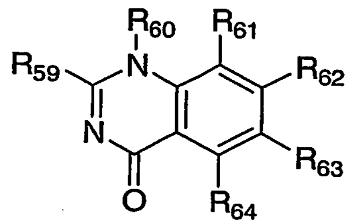
XII

wherein,

R_{58} is selected from the group consisting of substituted or unsubstituted aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl, and heterocyclylalkyl.

Preferably R_{58} is a pyranone substituent.

Quinazilinone compounds as described throughout this application include compounds of formula (XIII) as shown below:



XIII

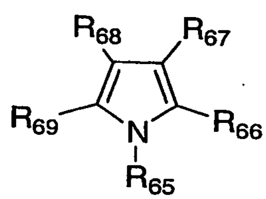
wherein,

R_{59} is selected from the group consisting of H, halogen, hydroxy, and substituted or unsubstituted alkyl, aminoalkyl, alklyaminoalkyl, alkoxy, dialkylaminoalkyl, hydroxyalkyl, alkenyl, alkynyl, carbocyclyl, carbocyclylalkyl, heterocyclyl, and heterocyclylalkyl;

R_{60} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, arylalkyl, heteroarylalkyl, and heterocyclylalkyl; and,

R_{61}, R_{62}, R_{63}, and R_{64} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Pyrrole compounds as described throughout this application include compounds of formula (XIV) as shown below:



XIV

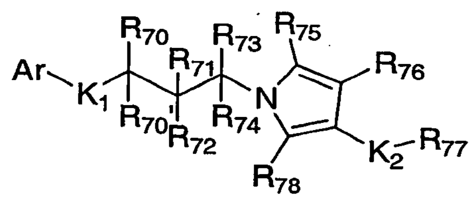
wherein,

R_{65} is selected from the group consisting of H, hydroxy, and substituted or unsubstituted alkyl, aryl, heteroaryl, heteroarylalkyl, arylalkyl, heteroarylaminoalkyl, arylaminoalkyl, heteroaryloxyalkyl, and aryloxyalkyl groups;

R_{66}, R_{67}, R_{68}, and R_{69} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and

substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Further preferred pyrrole compounds include those shown in Formula (XV):



(XV)

wherein:

K_1 is nitrogen, oxygen, or optionally substituted carbon;

W is absent or is selected from the group consisting of –O-, -S-, -S(O)-, $-SO_2-$, -NH-, -NH-CO-, -NR'CO-, $-NHSO_2-$, $-NR'SO_2-$, -CO-, $-CO_2-$, $-CH_2-$, $-CF_2-$, CHF, -CONH-, -CONR'-, and –NR'-, where R' is alkyl, substituted alkyl, cycloalkyl, aryl, heteroaryl, heterocyclo;

Ar is optionally substituted aryl, heteroaryl, or a protecting group;

R_{70} and R_{70}' are independently selected from the group consisting of hydrogen and methyl;

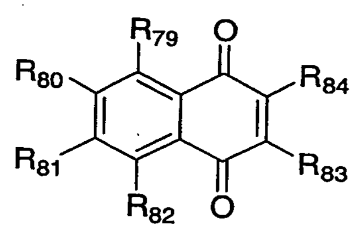
R_{71}, R_{72}, R_{73}, and R_{74} are independently selected from the group consisting of hydrogen, hydroxyl, and optionally substituted loweralkyl, cycloloweralkyl, cyclicaminoalkyl, alkylaminoalkyl, loweralkoxy, amino, alkylamino, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, aryl and heteroaryl;

R_{75} and R_{78} are independently selected from the group consisting of hydrogen, halo, and optionally substituted loweralkyl, cycloalkyl, alkoxy, amino, aminoalkoxy, carbonyloxy, aminocarbonyloxy, alkylcarbonylamino, arylcarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino, cycloimido, heterocycloimido, amidino, cycloamidino, heterocycloamidino, guanidinyl, aryl, heteroaryl, heterocycloalkyl, heterocyclocarbonyloxy, heteroarylcarbonyloxy, and arylsulfonamido;

R_{76} is selected from the group consisting of hydrogen, aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

R_{77} is selected from the group consisting of hydrogen, hydroxy, halo, carboxyl, nitro, amino, amido, amidino, imido, cyano, sulfonyl, methanesulonyl, and substituted or unsubstituted alkyl, alkoxy, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarbonyl, heteroaralkylcarbonyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, heteroarylcarbonyloxy, heteroaralkylcarbonyloxy, alkylaminocarbonyloxy, arylaminocarbonyloxy, formyl, loweralkylcarbonyl, loweralkoxycarbonyl, aminocarbonyl, aminoaryl, alkylsulfonyl, sulfonamido, aminoalkoxy, alkylamino, heteroarylamino, alkylcarbonylamino, alkylaminocarbonylamino, arylaminocarbonylamino, aralkylcarbonylamino, heteroarylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino cycloamido, cyclothioamido, cycloamidino, heterocycloamidino, cycloalkyl, cycloimido, heterocycloimido, guanidinyl, aryl, heteroaryl, heterocyclo, heterocycloalkyl, arylsulfonyl and arylsulfonamido;

Anthraquinone compounds of the instant invention include, for example, compounds of Formula (XVI):



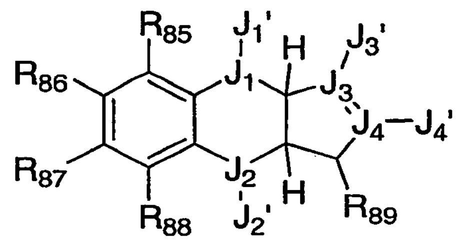
XVI

wherein,

R_{79}, R_{80}, R_{81}, and R_{82} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups; and,

R_{83} and R_{84} are taken together to form a substituted or unsubstituted 5-6 membered ring containing all carbon atoms or 1-2 heteroatoms selected from the group consisting of O, S, and N.

Quinoxaline compounds referred to throughout this application include tricyclic, partially unconjugated compounds optionally substituted with nitrogen heteroatoms as shown in the preferred quinoxaline embodiment (XVII) below:



XVII

wherein,

J_1 is either C or N,

J_1' is selected from the group consisting of H, substituted aryl, unsubstituted aryl, substituted heteroaryl, and unsubstituted heteroaryl;

J_2 is either C or N,

J_2' is selected from the group consisting of H, substituted aryl, unsubstituted aryl, substituted heteroaryl, and unsubstituted heteroaryl;

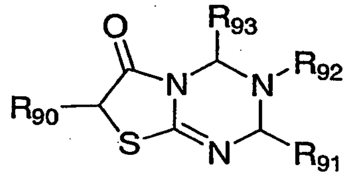
J_3 is selected from the group consisting of -CO-, -NH-, and -N=;

if J_4 is -O- then J_4' is absent; or,

if J_4 is =C- then J_4' is selected from the group consisting of H and substituted or unsubstituted alkyl, alkoxy, aryl, heteroaryl, heteroarylalkyl, arylalkyl, aminoalkyl, alkylamino, and alkylthio groups; and,

R_{85}, R_{86}, R_{87}, R_{88}, and R_{89} may be the same or different and are independently selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups.

Triazine compounds refer to substituted 6-membered heterocyclic groups with 3 nitrogen atoms distributed throughout the ring. The preferred embodiments of the instant invention include those shown in structures (XVIII), (XIX) and (XX) shown below:



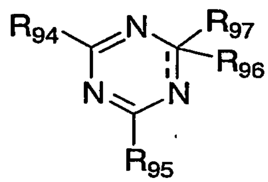
XVIII

wherein,

R_{90} is selected from the group consisting of substituted or unsubstituted alkyl, alkenyl, akynyl, aryl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, arylalkyl, and arylalkenyl;

R_{91} and R_{93} are independently selected from the group consisting of H, and unsubstituted alkyl;

R_{91} is aryl; preferably phenyl,



XIX

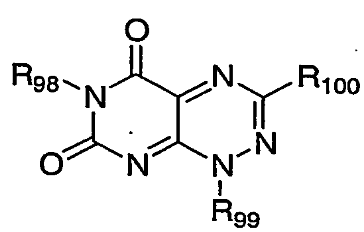
wherein,

R_{94} is selected from the group consisting of H, amino, alkyl, aminoalkyl, and halogen;

R_{95} is selected from the group consisting of substituted or unsubstituted aryl, arylamino, arylalkylamino, heteroaryl, heteroarylamino, and heteroalkylamino;

R_{96} and R_{97} are independently selected from the group consisting of H, halogen, and alkyl, preferably methyl; or,

R_{96} may form a double bond with the nitrogen atom directly below it as indicated by the dashed line in the above structure; and,



XX

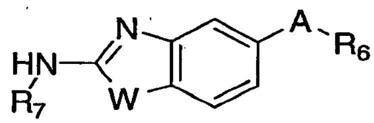
wherein,

R_{98} is selected from the group consisting of H, substituted alkyl, and unsubstituted alkyl; preferably methyl,

R_{99} is selected from the group consisting of H, substituted alkyl, and unsubstituted alkyl; preferably ethyl,

R_{100} is selected from the group consisting of substituted or unsubstituted aryl, heteroaryl, alkoxyaryl, arylalkyl, and heteroarylalkyl.

Benzazole compounds as described throughout this application include compounds of formula (XXI) as shown below:



XXI

wherein,

A is selected from the group consisting of -O-, -S-, -NH-, and $-NR_8$-;

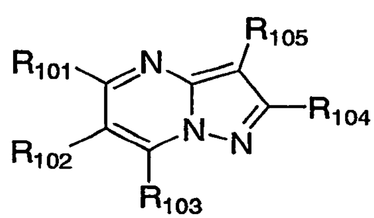
W is selected from the group consisting of $-CH_2$-, -O-, -S-, -NH-, and $-NR_8$-;

R_7 is selected from the group consisting of carbocyclyl, unfused carbocyclylcarbocyclyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted fused arylheteroaryl, unsubstituted fused arylheteroaryl, substituted unfused arylaryl and unsubstituted unfused arylaryl;

R_6 is selected from the group consisting of substituted or unsubstituted aryl, and heteroaryl; and,

R_8 is independently substituted or unsubstituted alkyl.

Pyrazalopyrimidine compounds as described throughout this application include compounds of formula (XXII) as shown below:



XXII

wherein,

R_{101} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, sulfonyl, aminosulfonyl, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{102} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{103} is selected from the group consisting of H, nitro, halogen, amino, hydroxy, cyano, carboxcyclic acid, trifluoromethyl, and substituted or unsubstituted alkyl, aryl, heteroaryl, alkoxy, alkylcarbonyl, alkylcarbonylamino, alkylaminocarbonyl, aminocarbonyl, arylalkoxy, heteroarylalkoxy, alkylamino, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, and carbocyclyl groups;

R_{104} is selected from the group consisting of H and substituted or unsubstituted aryl, heteroaryl, arylalkoxy, heteroarylalkoxy, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, carbocyclylalkyl and carbocyclyl groups;

R_{105} is selected from the group consisting of H and substituted or unsubstituted aryl, heteroaryl, arylalkoxy, heteroarylalkoxy, arylalkylamino, arylamino, heteroarylamino, heteroarylaminoalkyl, heterocyclyl, heterocyclylalkoxy, heterocyclylalkyl, carbocyclylalkyl and carbocyclyl groups;

wherein at least one of R_{104} and R_{105} is not H.

SMIP compounds identified by *in-vitro* (cellular or non-cellular assays) or *in-vivo* methods are thoroughly described in Methods 1 and 2 below.

Pharmaceutical compositions containing the compounds of the invention may be in any form suitable for the intended method of administration, including, for example, a solution, a suspension, or an emulsion. Liquid carriers are typically used in preparing solutions, suspensions, and emulsions. Liquid carriers contemplated for use in the practice of the present invention include, for example, water, saline, pharmaceutically acceptable organic solvent(s), pharmaceutically acceptable oils or fats, and the like, as well as mixtures of two or more thereof. The liquid carrier may contain other suitable pharmaceutically acceptable additives such as solubilizers, emulsifiers, nutrients, buffers, preservatives, suspending agents, thickening agents, viscosity regulators, stabilizers, and the like. Suitable organic solvents include, for example, monohydric alcohols, such as ethanol, and polyhydric alcohols, such as glycols. Suitable oils include, for example, soybean oil, coconut oil, olive oil, safflower oil, cottonseed oil, and the like. For parenteral administration, the carrier can also be an oily ester such as ethyl oleate, isopropyl myristate, and the like. Compositions of the present invention may also be in the form of microparticles, microcapsules, liposomal encapsulates, and the like, as well as combinations of any two or more thereof.

Other additives include immunostimulatory agents known in the art. Immunostimulatory oligonucleotides and polynucleotides are described in PCT WO 98/55495 and PCT WO 98/16247. U.S. Patent Application No. 2002/0164341 describes adjuvants including an unmethylated CpG dinucleotide (CpG ODN) and a non-nucleic acid adjuvant. U.S. Patent Application No. 2002/0197269 describes compositions comprising an antigen, an antigenic CpG-ODN and a polycationic polymer. Other immunostimulatory additives described in the art may be used, for example, as described in U.S. Patent No. 5,026,546; U.S. Patent No. 4,806,352; and U.S. Patent No. 5,026,543.

A controlled release delivery system may be used, such as a diffusion controlled matrix system or an erodible system, as described for example in: Lee, "Diffusion-Controlled Matrix Systems", pp. 155-198 and Ron and Langer, "Erodible Systems", pp. 199-224, in "Treatise on Controlled Drug Delivery", A. Kydonieus Ed., Marcel Dekker, Inc., New York 1992. The matrix may be, for example, a biodegradable material that can degrade spontaneously *in situ* and *in vivo* for, example, by hydrolysis or enzymatic cleavage, *e.g.*, by proteases. The delivery system may be, for example, a naturally occurring or synthetic polymer or copolymer, for example in the form of a hydrogel. Exemplary polymers with cleavable linkages include polyesters, polyorthoesters, polyanhydrides, polysaccharides, poly(phosphoesters), polyamides, polyurethanes, poly(imidocarbonates) and poly(phosphazenes).

The compounds of the invention may be administered enterally, orally, parenterally, sublingually, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. For example, suitable modes of administration include oral, subcutaneous, transdermal, transmucosal, iontophoretic, intravenous, intramuscular, intraperitoneal, intranasal, subdermal, rectal, and the like. Topical administration may also involve the use of transdermal administration such as transdermal patches or ionophoresis devices. The term parenteral includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-propanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavoring, and perfuming agents.

As to the mode of administration, it should be emphasized that it is the combination of therapeutic agents that gives rise to its synergistic therapeutic effect no matter whether the first and the second agent are administered together or separately. Therefore, the two agents may be given together in a single dose or in separate ones with respect to space and time.

Effective amounts of the compounds of the invention generally include any amount sufficient to detectably treat viral infections.

Successful treatment of a subject in accordance with the invention may result in the inducement of a reduction or alleviation of symptoms in a subject afflicted with a medical or biological disorder to, for example, halt the further progression of the disorder, or the prevention of the disorder.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. The therapeutically effective amount for a given situation can be readily determined by routine experimentation and is within the skill and judgment of the ordinary clinician.

The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.W., p. 33 *et seq* (1976).

While the SMIP compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other agents used in the treatment of SARSs. Other representative agents useful in combination with the

compounds of the invention for the treatment of viral infections include, for example, interferon, ribavirin, gancyclovir and the like.

When additional active agents are used in combination with the compounds of the present invention, the additional active agents may generally be employed in therapeutic amounts as indicated in the PHYSICIANS' DESK REFERENCE (PDR) 53rd Edition (1999), that is incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art.

The compounds of the invention and the other therapeutically active agents can be administered at the recommended maximum clinical dosage or at lower doses. Dosage levels of the active compounds in the compositions of the invention may be varied so as to obtain a desired therapeutic response depending on the route of administration, severity of the disease and the response of the patient. The combination can be administered as separate compositions or as a single dosage form containing both agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Compounds of the present invention can be readily synthesized using the methods described herein, or other methods, that are well known in the art..

The compounds can be used in the form of salts derived from inorganic or organic acids. These salts include but are not limited to the following: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-napthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, p-toluenesulfonate and undecanoate. Also, the basic nitrogen-containing groups can be quaternized with such agents as loweralkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides, and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl, and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides, and others. Water or oil-soluble or dispersible products are thereby obtained.

Examples of acids that may be employed to form pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid and such organic acids as oxalic acid, maleic acid, succinic acid and citric acid. Basic

addition salts can be prepared *in situ* during the final isolation and purification of the compounds of formula (I), or separately by reacting carboxylic acid moieties with a suitable base such as the hydroxide, carbonate or bicarbonate of a pharmaceutical acceptable metal cation or with ammonia, or an organic primary, secondary or tertiary amine. Pharmaceutical acceptable salts include, but are not limited to, cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, aluminum salts and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Other representative organic amines useful for the formation of base addition salts include diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

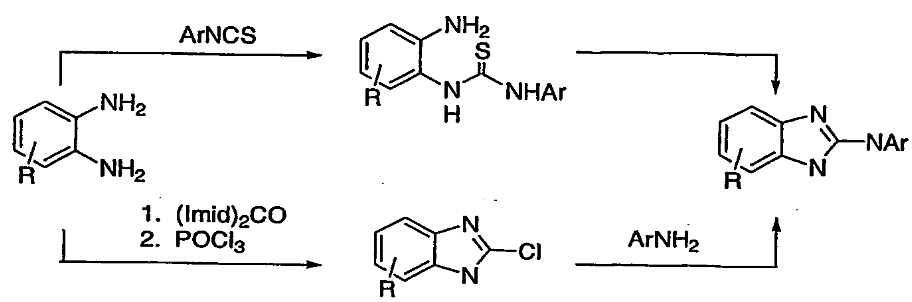
Various compounds and methods of their synthesis are disclosed in international patent application Publication Nos. WO02/18327 (benzamide and pyridylamide based compounds); WO0222598, and WO02/18383 (ABIQ based compounds); and WO 02/81443 (pthalamide base compounds), that have been found within context of this invention to be useful for immune potentiation. The entire disclosure of these U.S. and international publications is incorporated herein by this reference. Other compounds or intermediates of interest in the present invention were purchased from commercially available sources using the following method: the chemical structure of interest was drawn into the ACD-SC database (from MDL Information Systems). A search of the following companies/institutions, among others, retrieved the identified compound's supplier and purchasing information: ASDI, ASINEX, BIONET, CHEMBRIDGE, CHEMDIV, CHEMEX, CHEMSTAR, COMGENEX, CSC, INTERBIOSCREEN, LABOTEST, MAYBRIDGE, MICROSOURCE/GENESIS, OLIVIA, ORION, PEAKDALE, RYAN SCIENTIFIC, SPECS, TIMTEC, U OF FLORIDA, and ZELINSKY.

BENZAZOLE COMPOUNDS

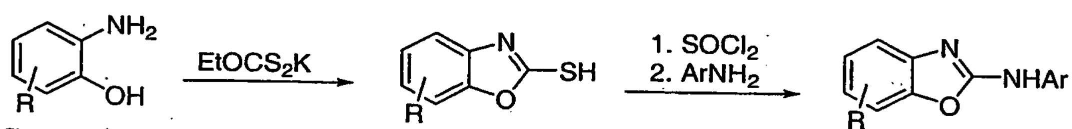
Scheme 1

Compounds of the invention containing a benzimidazole core may be prepared using a number of methods familiar to one of skill in the art. In one method, suitably functionalized diamines may be coupled with various thioisocyanates to form the intermediate thioureas. Cyclization to form the benzimidazole moiety may be effected under known conditions such as with treatment carbodiimides or alkyl halides. Alternatively the diamines may be reacted

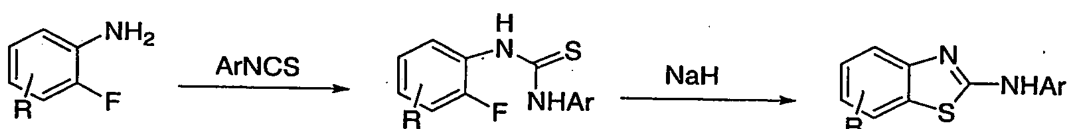
sequentially with carbonyl diimidazole and phosphoryl chloride followed by coupling with the appropriate amine.



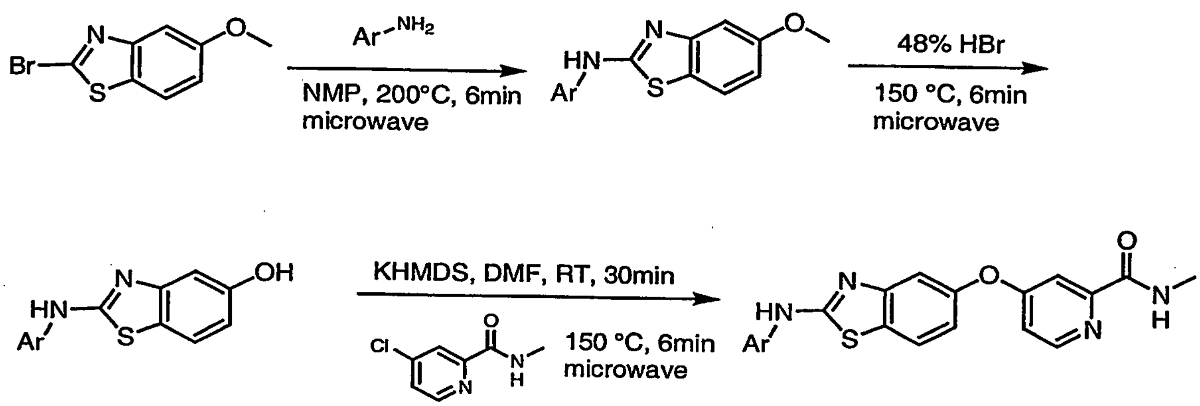
Compounds containing the oxazole structure may similarly be prepared according to the methods above or according to other known general procedures. Haviv et. al. (J. Med. Chem. 1988, 31, 1719) describes a procedure for assembling oxazole cores wherein a hydroxy aniline is treated with ethyl potassium xanthate. The resulting sulfuryl benzoxazole may then be chlorinated and coupled with an amine.



Compounds containing a benzothiazole core may also be prepared according to known methods. An ortho-halothioisocyanate may be reacted with an amine to form a thiourea. Reduction with NaH then allows formation of the thiazole ring.

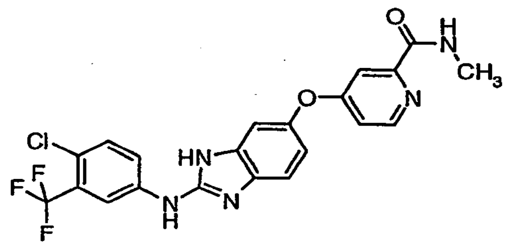


Benzothiazoles may generally be substituted in accordance with the present invention, such as through the following synthetic pathway:



Synthesis of 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide

The compound 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide (159322) was synthesized as follows:



Step 1. Synthesis of 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide: A mixture containing 4-amino-3-nitrophenol (1eq) and potassium bis(trimethylsilyl)amide (2eq) was stirred in dimethylformamide for 2 hours at room temperature. To this mixture was added (4-chloro(2-pyridyl))-*N*-methylcarboxamide (1eq) and potassium carbonate (1.2eq) and stirred at 90°C for 3 days. The reaction mixture was then concentrated and partitioned between ethyl acetate and water. The organic layer was separated and washed with brine, dried, filtered, and concentrated in vacuum to give brown solid. Purification on silica gel (2% triethyl amine / 50% ethyl acetate in hexane) gave 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide as an orange solid. The product gave satisfactory NMR. HPLC, 3.39min; MS: $MH^+ = 289$.

Step 2. Synthesis of 4-[(3,4-diaminophenyl)oxy]-*N*-methylpyridine-2-carboxamide: The mixture containing [4-(3-amino-4-nitrophenoxy)(2-pyridyl)]-*N*- in methanol with catalytic amount of 10%Pd/C was hydrogenated until disappearance of the yellow color to yield the product amine. HPLC, 2.5mins; MS: $MH^+ = 259$.

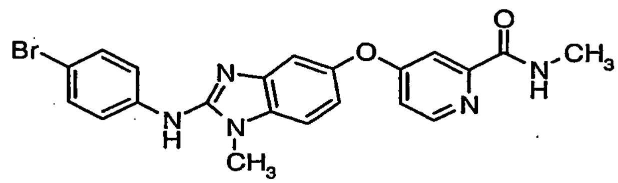
Step 3. Synthesis of 4-[(2-{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide:

The mixture containing 4-[(3,4-diaminophenyl)oxy]-*N*-methylpyridine-2-carboxamide (1eq) and 4-chloro-3-(trifluoromethyl)benzeneisothiocyanate (1eq) in tetrahydrofuran was stirred at room temperature for 16 hours to give the corresponding thiourea. To the resulting mixture was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2eq) and the mixture was stirred for another 10 hours. The mixture was concentrated and partitioned between ethyl acetate and water. The organic layer was washed with brine and dried. Purification on HPLC gave 4-[(2-

{[4-chloro-3-(trifluoromethyl)phenyl]amino}-1H-benzimidazol-6-yl)oxy]-N-methylpyridine-2-carboxamide. MS: MH^+ = 462

Synthesis of 4-({2-[(4-bromophenyl)amino]-1-methyl-
1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide

The compound 4-({2-[(4-bromophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide (161651) was synthesized as follows:



Step 1. Synthesis of 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide: A solution of 4-[(4-amino-3-nitrophenyl)oxy]-*N*-methylpyridine-2-carboxamide (1eq) in methylene chloride was treated with trifluoroacetic anhydride (1eq) and stirred for 10 minutes at 0 °C. The mixture was quenched with satd. $NaHCO_3$ solution. The organic layer was separated and washed with water, brine, dried and evaporated. MS: MH^+=385.2

To a solution of the trifluroacetamide (1eq) in a mixture of toluene, acetonitrile and sodium hydroxide solution (50%) was added benzyltrimethylammonium chloride (1eq) and dimethyl sulfate (1.2eq). The biphasic mixture was stirred overnight at room temperature and evaporated. The mixture was taken up in ethyl acetate, washed with water, brine, dried and evaporated. The crude product was purified by column chromatography eluting with 1:1 hexanes and ethylacetate followed by 2% triethylamine in 1:1 hexanes and ethyl acetate followed by 2% triethylamine in 1:1 hexanes and ethyl acetate to afford *N*-methyl-4-{[4-(methylamino)-3-nitrophenyl]oxy}pyridine-2-carboxamide as a reddish orange solid. MS: MH^+ = 303.1.

The solution of nitromethylaniline in methanol was treated with 5% palladium on carbon and stirred under hydrogen atmosphere for 15 min. (until the disappearance of yellow coloration) at room temperature. The mixture was filtered and the filtrate was concentrated to provide 0.36 g of the diamine 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide. MS: MH^+ = 273.3.

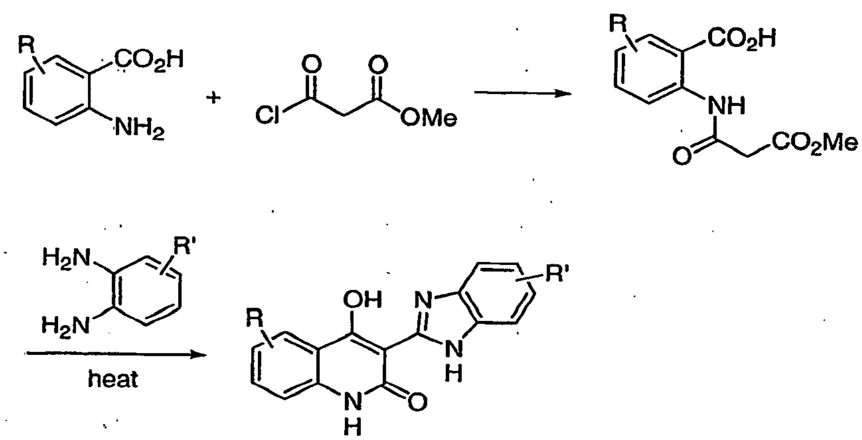
Step 2. Synthesis of 4-({2-[(4-bromophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide: A solution of the diamine 4-{[3-amino-4-(methylamino)phenyl]oxy}-*N*-methylpyridine-2-carboxamide (1eq) in methanol was treated with 4-bromophenylisothiocyanate (1eq) and stirred at 60 °C - 65°C for 2 hours. The reaction mixture was cooled down to room temperature and methyl iodide (1eq) was added and stirred overnight at 60°C. The reaction was cooled to room temperature, evaporated, taken up in ethyl

acetate, and washed with water and brine, dried, and evaporated under reduced pressure. Column chromatography using a gradient solvent system of hexanes and ethyl acetate and either 1:1 methylene chloride and acetone or 5% methanol in methylene chloride yielded the product as a half white powder. MS: MH^+=452.3

AMINOBENZIMIDAZOLYLQUINOLINONES

Compounds of structure I may be synthesized from simple starting molecules as shown in Schemes 1-4 and exemplified in the Examples. As shown in Scheme 1, compounds of structure I may generally be prepared using aromatic compounds substituted with amines and carboxylic acid groups.

Scheme 2.

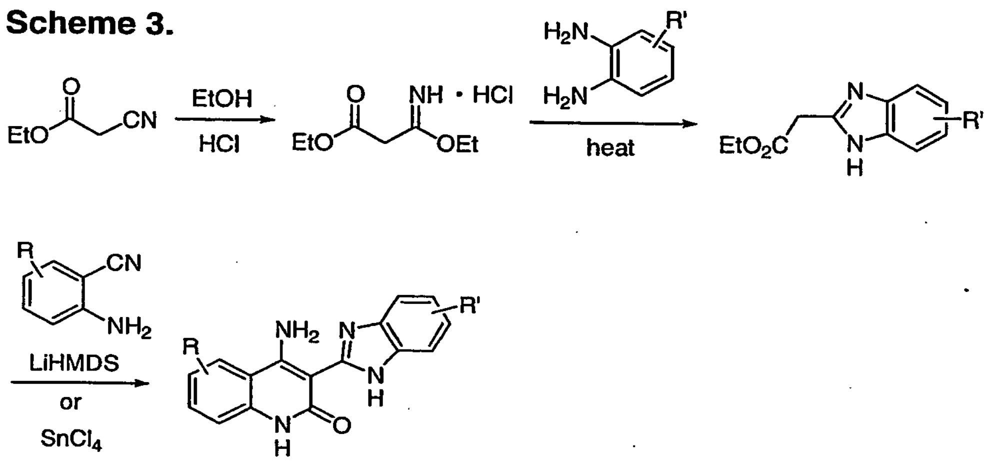


As shown in Scheme 2, a substituted aromatic compound such as a substituted or unsubstituted 2-aminobenzoic acid may be reacted with an acyl halide such as methyl 2-(chlorocarbonyl)acetate to produce an amide that will react with a substituted or unsubstituted 1,2-diaminobenzene. The resulting product is a 4-hydroxy-substituted compound of structure I. One skilled in the art will recognize that the procedure set forth in Scheme 1 may be modified to produce various compounds.

A method for preparing 4-amino substituted compounds of structure I is shown in Scheme 3. As shown in Scheme 3, aromatic compounds substituted with amine and nitrile groups may be used to synthesize 4-amino substituted compounds of structure I. A compound such as ethyl 2-cyanoacetate may be reacted with ethanol to produce ethyl 3-ethoxy-3-iminopropanoate hydrochloride. Subsequent reaction with a substituted or unsubstituted 1,2-phenylenediamine provides substituted or unsubstituted ethyl 2-benzimidazol-2-ylacetate. Reaction of a substituted or unsubstituted ethyl 2-benzimidazol-2-ylacetate with an aromatic

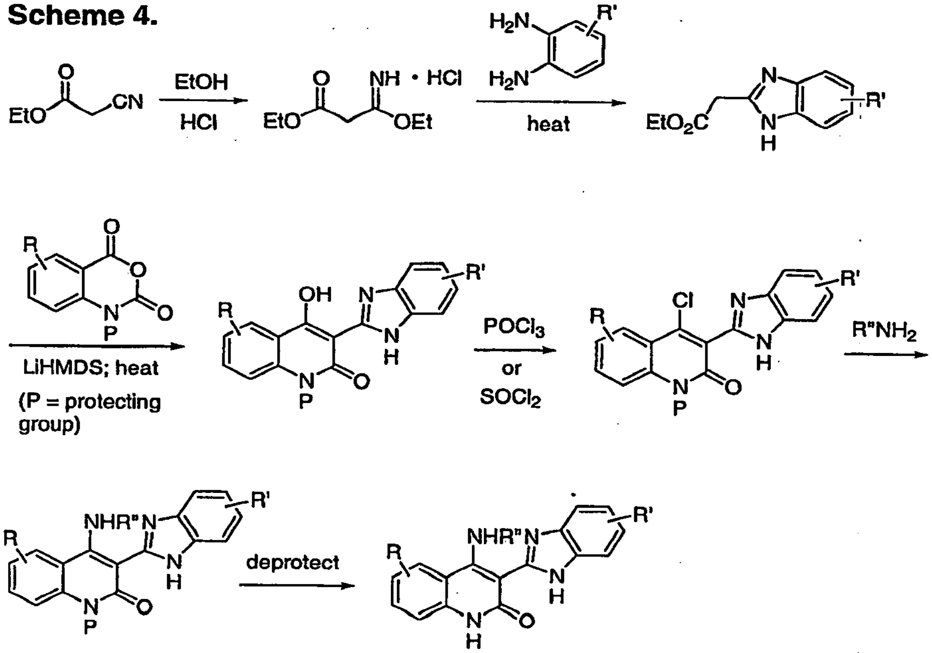
compound having an amine and nitrile group such as substituted or unsubstituted 2-aminobenzonitrile with a base such as lithium bis(trimethylsilyl)amide or a Lewis acid such as tin tetrachloride provides the substituted or unsubstituted 4-amino substituted compound of structure I.

Scheme 3.



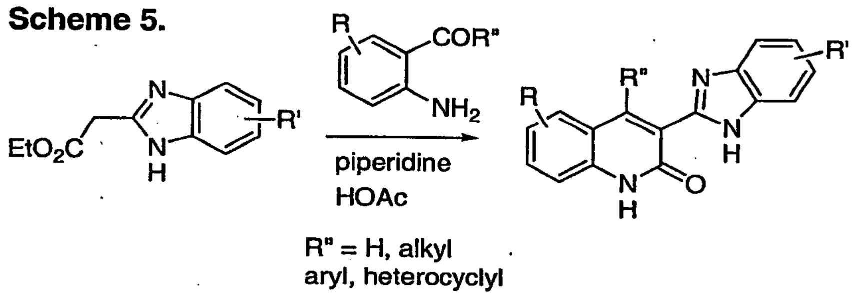
Scheme 4 illustrates a general synthetic route that allows for the synthesis of 4-dialkylamino and 4-alkylamino compounds of structure I. An inspection of Scheme 3 shows that 4-hydroxy substituted compounds of structure I may be converted into the 4-chloro derivative by reaction with phosphorous oxychloride or thionyl chloride. The 4-chloro derivative may then be reacted with an alkylamine or dialkylamine to produce the corresponding 4-alkylamino or 4-dialkylamino derivative. Deprotection affords the final 4-alkylamino or 4-dialkylamino compounds of structure I. Other groups that may be reacted with the 4-chloro derivative in this manner include, but are not limited to, ROH, RSH, and CuCN.

Scheme 4.



As shown in Scheme 5, the synthesis of compounds of structure I having a H, alkyl group, aryl group, or heterocyclyl group in the 4-position may be accomplished using a substituted or unsubstituted 2-benzimidazol-2-ylacetate prepared as shown in Schemes 3 and 4.

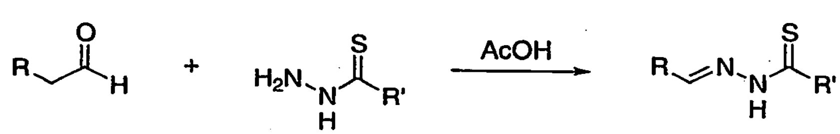
Scheme 5.



THIOSEMCARBAZONES

General procedure for the preparation of thiosemicarbazones

Scheme 6



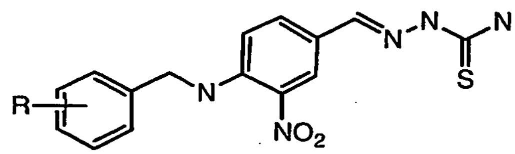
A solution of aldehyde (1.0 equiv.) and thiosemicarbazide (1.05 equiv.) in acetic acid was stirred overnight. Excess of acetic acid was removed to give a residue, that was washed with ethanol, or purified by preparative-HPLC to give the thiosemicarbazone.

Scheme 7

A solution of aldehyde (1.0 equiv.), thiosemicarbazide (1.05 equiv.) and acetic acid (0.1 equiv.) in methanol was stirred overnight. Methanol was removed to give a residue, that was worked up as in Scheme 6.

Scheme 8

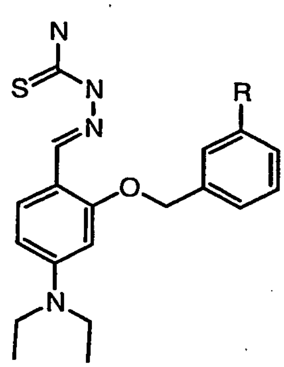
To a solution of {[(1E)-1-aza-2-(4-fluoro-3-nitrophenyl)vinyl]amino}-aminomethane-1-thione in ethanol was added an arylamine (2.1 equiv.). The solution was stirred at room temperature until the starting fluoride disappeared. The solution was purified to the product.



Scheme 9

A mixture of 4-(diethylamino)-2-hydroxybenzaldehyde (1 equiv.), benzylic bromide (1.2 equiv.) and powder potassium carbonate in ethanol was stirred at room temperature for 2 days. Ethanol was removed, and the residue was dissolved in ethyl acetate and water. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4., and concentrated. The residue was purified on silica gel eluting with ethyl acetate/hexane to give 4-(diethylamino)-2-benzoxylic-benzaldehyde.

The aldehydes were converted to thiosemicarbazones according to Scheme 7.



Scheme 10

A solution of 3,4-difluorobenzenecarbonitrile (1 equiv.), amine (1.5 equiv.) and DIEA (2 equiv.) in NMP was heated in a Smith Microwave (Personal Chemistry) for 30 minutes. The reaction mixture was purified on silica gel to give 4-substituted 3-fluorobenzenecarbonitrile.

To a solution of nitrile in toluene at –78 °C was added DIBAL-H (1 M in toluene, 1.5 equiv.). The reaction mixture was warmed to rt, and stirred for 16 h, and quenched with methanol/ethyl acetate/brine (1:1:4). After being stirred at rt for 30 min, the solution was extracted with ethyl acetate (3x). The combined organic layers were washed with aqueous $NaHCO_3$, brine and concentrated. The aldehyde was purified on silica gel or directly converted to thiosemicarbazones (Scheme 7).

Scheme 11

A solution of 2,4,5-trifluorobenzenecarbonitrile (1 equiv.) and 4-arylpiperazine (1.2 equiv.) and DIEA (1.2 equiv.) in THF was heated at 80 °C for 2 hours. The mixture was purified on silica gel to give 4-substituted 2,5-difluorobenzenecarbonitrile.

Scheme 12

To an alcohol (1.0 equiv) was added potassium *t*-butoxide in THF (1 M, 1.1 equiv). After 5 minutes, the solution was added to a solution of 4-N-substituted-2,5-difluorobenzenecarbonitrile (1 equiv.) in THF. The reaction mixture was stirred at rt overnight and quenched with aqueous ammonium chloride. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with brine, and concentrated to give a residue, that was purified to give 4-N-substituted-2-O-substituted-5-fluorobenzenecarbonitrile.

4-N-substituted-2-O-substituted-5-fluorobenzenecarbonitrile was reduced with DIBAL-H to give a 4-N-substituted-2-O-substituted-5-fluorobenzaldehyde according to procedure in Scheme 10.

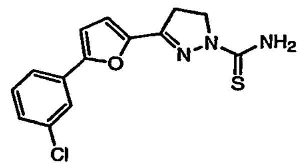
The aldehyde was converted to the corresponding thiosemicarbazone using Scheme 7.

Scheme 13

A solution of 4-N-substituted-2,5-difluorobenzenecarbonitrile (1 equiv.), amine (1.5 equiv.) and DIEA (2 equiv.) in NMP was heated in a Smith Microwave (Personal Chemistry) for 30 minutes. The reaction mixture was purified on silica gel to give 4-N-substituted-2-N-substituted-5-fluorobenzenecarbonitrile.

4-N-substituted-2-N-substituted-5-fluorobenzenecarbonitrile was reduced with DIBAL-H according to procedure described in Scheme 10 to give 4-N-substituted-2-N-substituted-5-fluorobenzaldehyde.

Preparation of amino{3-[5-(3-chlorophenyl)(2-furyl)](2-pyrazolinyl)}methane-1-thione



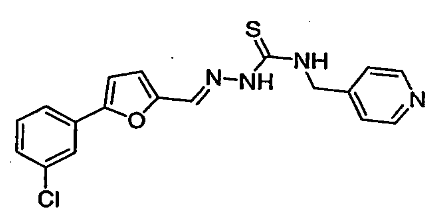
To a solution of 5-(3-chlorophenyl)furan-2-carbaldehyde (1.0 equiv.) in THF at 0 °C was added MeMgBr in ether (3.0 equiv.) and stirred for 45 min. The reaction was quenched with water, diluted with ether and filtered through Celite. The organic layer was separated and washed with brine, dried over $MgSO_4$, and concentrated to give the 1-[5-(3-chlorophenyl)-2-furyl]ethan-1-ol.

To a solution of secondary alcohol(1.0 equiv.) in CH_2Cl_2 was added MnO_2 (10 equiv.). The reaction was stirred overnight, filtered through Celite, and concentrated to give 1-[5-(3-chlorophenyl)-2-furyl]ethan-1-one.

To a mixture of ketone (1.0 equiv.), paraformaldehyde (2.0 equiv.), and dimethylamine hydrochloride (2.0equiv) and molecular sieves in ethanol was added concentrated hydrochloric acid (cat.). The reaction was refluxed overnight under nitrogen and the concentrated. A few drops of HCl was added, and the mixture was worked up with DCM and water. The organic layer was discarded. The aqueous layer was adjusted to basic and extracted with DCM (3x). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to yield 3-(dimethylamino)-1-[5-(3-chlorophenyl)(2-furyl)]propan-1-one.

Thiosemicarbazide (1.0 equiv.) was dissolved in MeOH upon heating under nitrogen. Aqueous sodium hydroxide (6 M, 9.0 equiv.) was added to the reaction. A methanol solution of 3-(dimethylamino)-1-[5-(3-chlorophenyl)(2-furyl)]propan-1-one (1.0 equiv) was then added dropwise to the reaction mixture. The solvent was removed and the residue was dissolved in DCM and washed with water, brine, dried over $MgSO_4$, and concentrated. The final compound was purified by preparative-HPLC to give amino{3-[5-(3-chlorophenyl)(2-furyl)](2-pyrazolinyl)}methane-1-thione; LC/MS m/z 306.2 (MH+); Rt =3.06 minutes .

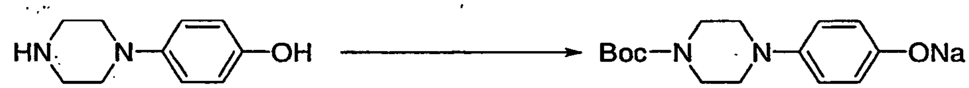
Scheme 14



To a solution of 4-pyridylmethylamine (1.0 equiv.) and triethylamine (2.0 equiv.) in $CHCl_3$ was added CS_2 (1.0 equiv.)) and stirred overnight. The reaction was cooled to 0 °C and ethyl chloroformate (1.0 equiv.) was added dropwise. The reaction was stirred for 15 min at 0 °C and then stirred at room temperature for 2 hrs followed by addition of (tert-butyl)oxycarbohydrazide (1.2 equiv.). After stirring for an addition hour the mixture was washed with aqueous citric acid (5%), saturated $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated. The desired Boc protected thiosemicarbazide was purified using column chromatography.

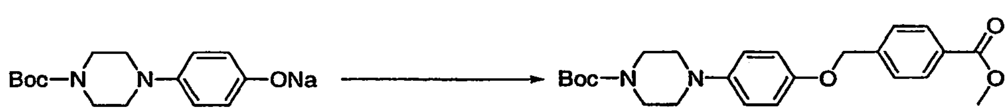
To a solution of Boc protected thiosemicarbazide (1.0 equiv.) dissolved in DCM was added HCl in dioxane (2M, 8.3 equiv.) and stirred for 15 min. MeOH is then added to dissolve the precipitate, followed by addition of the furfural, and small amount of acetic acid (0.5 mL). The mixture is stirred overnight and the solvents are removed to give a residue purified by preparative-HPLC to give the thiosemicarbazone.

Synthesis of 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzaldehyde



To a solution of 4-piperazin-1-yl phenol (1 equivalent) in $CHCl_3$, cooled to 0 °C, was added di-*t*-butyl dicarbonate (1 equivalent) in $CHCl_3$ drop-wise. The solution was stirred at 0 °C for 1 hour before removing from the cold bath and stirring at ambient temperatures for 18 hours. The organic solution was washed aqueous $NaHCO_3$ and brine dried over $MgSO_4$ and concentrated the crude material was used without purification.

A solution of the resulting 4-(1-BOC-piperazin-4-yl)phenol (1 equivalent) in dry CH_3CN was slowly added drop-wise to a slurry of NaH (1 equivalent) in dry CH_3CN at room temperature under N_2. The slurry was stirred at room temperature for 2 hours before the solids were filtered and washed with Et_2O.



Sodium 4-(1-BOC-piperazin-4-yl)phenoxide (1 equivalent) and methyl 4-bromomethylbenzoate (1 equivalent) were combined in dry acetone and heated to reflux at 60 °C for 18 hours. The slurry was filtered and the filtrate was then concentrated to provide the crude methyl 4-[4-(1-BOC-piperazin-4-yl)phenoxymethyl]benzoate, that was used without purification.

having sequence identity to an amino acid sequence selected from the group consisting of the sequences shown in Figure 127, and in particular SEQ ID NOs: 10506 to 10570.

The invention also provides fragments of amino acid sequences encoded by SEQ ID NO: 10505. The invention also provides fragments of amino acid sequences selected from the group consisting of SEQ ID NOs: 10506 to 10570. In one embodiment, the fragment does not consist entirely of a known amino acid sequence of a SARS virus or a known amino acid sequence of a coronavirus.

In one embodiment, the invention includes a polypeptide comprising an amino acid sequence from the 5'3' Frame 3 of Figure 127. Some encoded open reading frames within this translation are: SEQ ID NO: 10533; SEQ ID NO: 10571; SEQ ID NO: 10572; SEQ ID NO: 10573; SEQ ID NO: 10574.

The invention includes a polypeptide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10533, SEQ ID NO: 10571, SEQ ID NO: 10572, SEQ ID NO: 10573 and SEQ ID NO: 10574. The invention includes a polypeptide having sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO: 10533, SEQ ID NO: 10571, SEQ ID NO: 10572, SEQ ID NO: 10573 and SEQ ID NO: 10574. The invention includes a fragment of a polypeptide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10533, SEQ ID NO: 10571, SEQ ID NO: 10572, SEQ ID NO: 10573 and SEQ ID NO: 10574.

Partial BLAST results of SEQ ID NO: 10533 against GenBank are given below:

```
>gi|7739601|gb|AAF68926.1|AF207902_11 nucleocapsid protein [murine
hepatitis virus strain ML-11]
          Length = 451

 Score =  147 bits (370), Expect = 3e-34
 Identities = 102/252 (40%), Positives = 137/252 (54%), Gaps = 18/252 (7%)

Query: 49  SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
            SWF+ +TQ  K +E +F +GQGVPI +       +Q GY+ R  RR  +  DG+ K+L PRW
Sbjct: 63  SWFSGITQFQKGKEFQFAQGQGVPIASGIPASEQKGYWYRHNRRSFKTPDGQHKQLLPRW 122

Query: 107 YFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP 166
            YFYYLGTGP A    YG + EG+VWVA++ A          + R+P+++ A   +   GT LP
Sbjct: 123 YFYYLGTGPHAGAEYGDDIEGVVWVASQQADTKTTADVVERDPSSHEAIPTRFAPGTVLP 182

Query: 167 KGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRLN 226
            +GFY EGS    + AS   S          N   SS     PA              +A L+L +L
Sbjct: 183 QGFYVEGSGRSAPASRSGSRSQSRGPNNRARSSSNQRQPASAVKPDMAEEIAALVLAKLG 242

Query: 227 QLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQTQG 282
            +         GQ +Q    VTK+SA E   +   KPRQKRT   KQ   V Q FG+RGP Q
Sbjct: 243 K------DAGQPKQ---VTKQSAKEVRQKILTKPRQKRTPNKQCPVQQCFGKRGPNQ--- 290

Query: 283 NFGDQDLIRQGT 294
            NFG    ++++ GT
Sbjct: 291 NFGGSEMLKLGT 302
```

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>gi|3132999|gb|AAC16422.1|    nucleocapsid protein [murine hepatitis virus
strain 2]
          Length = 451

 Score =  147 bits (370), Expect = 3e-34
 Identities = 102/252 (40%), Positives = 137/252 (54%), Gaps = 18/252 (7%)

Query: 49   SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
            SWF+ +TQ  K +E +F +GQGVPI +       +Q GY+ R  RR  +  DG+ K+L PRW
Sbjct: 63   SWFSGITQFQKGKEFQFAQGQGVPIASGIPASEQKGYWYRHNRRSFKTPDGQHKQLLPRW 122

Query: 107  YFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP 166
            YFYYLGTGP A   YG + EG+VWVA++ A          +  R+P+++ A   +   GT LP
Sbjct: 123  YFYYLGTGPHAGAEYGDDIEGVVWVASQQADTKTTADVVERDPSSHEAIPTKFAPGTVLP 182

Query: 167  KGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRLN 226
            +GFY EGS   + AS   S          N   SS    PA          +A L+L +L
Sbjct: 183  QGFYVEGSGKSAPASRSGSRSQSRGPNNRARSSSNQRQPASAVKPDMAEEIAALVLAKLG 242

Query: 227  QLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQTQG 282
            +          GQ +Q    VTK+SA E  +     KPRQKRT  KQ  V Q FG+RGP Q
Sbjct: 243  K------DAGQPKQ---VTKQSAKEVRQKILTKPRQKRTPNKQCPVQQCFGKRGPNQ--- 290

Query: 283  NFGDQDLIRQGT 294
            NFG  ++++ GT
Sbjct: 291  NFGGSEMLKLGT 302


>gi|127877|sp|P03417|NCAP_CVMJH  Nucleocapsid protein
 gi|74859|pir||VHIHMJ    nucleocapsid protein - murine hepatitis virus
(strain JHM)
 gi|58973|emb|CAA25497.1|   nucleocapsid protein [Murine hepatitis virus]
          Length = 455

 Score =  146 bits (369), Expect = 4e-34
 Identities = 110/254 (43%), Positives = 142/254 (55%), Gaps = 22/254 (8%)

Query: 49   SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
            SWF+ +TQ  K +E +F +GQGVPI         Q GY+ R  RR  +  DG+ K+L PRW
Sbjct: 67   SWFSGITQFQKGKEFQFAQGQGVPIANGIPASQQKGYWYRHNRRSFKTPDGQQKQLLPRW 126

Query: 107  YFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP 166
            YFYYLGTGP A   YG + EG+VWVA++ A          I  R+P+++ A   +   GT LP
Sbjct: 127  YFYYLGTGPYAGAEYGDDIEGVVWVASQQAETRTSADIVERDPSSHEAIPTRFAPGTVLP 186

Query: 167  KGFYAEGSRGGSQASSRSSSR--SRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDR 224
            +GFY EGS G S  +SRS SR  SRG   N    SS    PA          +A L+L +
Sbjct: 187  QGFYVEGS-GRSAPASRSGSRPQSRG-PNNRARSSSNQRQPASTVKPDMAEEIAALVLAK 244

Query: 225  LNQLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQT 280
            L +          GQ +Q    VTK+SA E  +     KPRQKRT  KQ  V Q FG+RGP Q
Sbjct: 245  LGK------DAGQPKQ---VTKQSAKEVRQKILNKPRQKRTPNKQCPVQQCFGKRGPNQ- 294

Query: 281  QGNFGDQDLIRQGT 294
              NFG  ++++ GT
Sbjct: 295  --NFGGPEMLKLGT 306
```

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>gi|6625766|gb|AAF19389.1|AF201929_7      nucleocapsid   protein   [murine
hepatitis virus strain 2]
 gi|7769348|gb|AAF69338.1|AF208066_11      nucleocapsid   protein   [murine
hepatitis virus]
          Length = 451

 Score =  146 bits (368), Expect = 5e-34
 Identities = 102/252 (40%), Positives = 137/252 (54%), Gaps = 18/252 (7%)

Query: 49    SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
             SWF+ +TQ  K +E +F +GQGVPI +     +Q GY+ R  RR  +  DG+ K+L PRW
Sbjct: 63    SWFSGITQFQKGKEFQFAQGQGVPIASGIPASEQKGYWYRHNRRSFKTPDGQHKQLLPRW 122

Query: 107   YFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLP 166
             YFYYLGTGP A   YG + EG+VWVA++ A       +  R+P+++ A   +   GT LP
Sbjct: 123   YFYYLGTGPHAGAEYGDDIEGVVWVASQQADTKTTADVVERDPSSHEAIPTRFAPGTVLP 182

Query: 167   KGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRLN 226
             +GFY EGS   + AS   S        N    SS    PA          +A L+L +L
Sbjct: 183   QGFYVEGSGRSAPASRSGSRSQSRGPNNRARSSSNQRQPASAVKPDMAEEIAALVLAKLG 242

Query: 227   QLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQTQG 282
             +        GQ +Q   VTK+SA E  +    KPRQKRT  KQ  V Q FG+RGP Q
Sbjct: 243   K------DAGQPKQ---VTKQSAKEVRQKILTKPRQKRTPNKQCPVQQCFGKRGPNQ--- 290

Query: 283   NFGDQDLIRQGT 294
             NFG  ++++ GT
Sbjct: 291   NFGGSEMLKLGT 302


>gi|21734854|gb|AAM77005.1|AF481863_7   phosphorylated nucleocapsid protein
N [porcine hemagglutinating encephalomyelitis virus]
          Length = 449

 Score =  145 bits (366), Expect = 8e-34
 Identities = 107/253 (42%), Positives = 145/253 (57%), Gaps = 18/253 (7%)

Query: 49    SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
             SWF+ +TQ  K +E  F  GQGVPI       +  GY+ R  RR  +  DG  ++L PRW
Sbjct: 64    SWFSGITQFQKGKEFEFAEGQGVPIAPGVPATEAKGYWYRHNRRSFKTADGNQRQLLPRW 123

Query: 107   YFYYLGTGPEASLPYGANKEGIVWVATEGA-LNTPKDHIGTRNPNNNAATVLQLPQGTTL 165
             YFYYLGTGP A   YG + +G+ WVA+  A +NTP D I  R+P+++ A   + P GT L
Sbjct: 124   YFYYLGTGPHAKHQYGTDIDGVFWVASNQADINTPAD-IVDRDPSSDEAIPTRFPPGTVL 182

Query: 166   PKGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRL 225
             P+G+Y EGS G S  +SRS+SR+  N   S    SR NS  R ++ G    +A    D++
Sbjct: 183   PQGYYIEGS-GRSAPNSRSTSRA-PNRAPSAGSRSRANSGNRTSTPGVTPDMA----DQI 236

Query: 226   NQLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQTQ 281
               L     GK    + Q VTK++A E  +    KPRQKR+  KQ  V Q FG+RGP Q
Sbjct: 237   ASLVLAKLGK-DATKPQQVTKQTAKEVRQKILNKPRQKRSPNKQCTVQQCFGKRGPNQ-- 293

Query: 282   GNFGDQDLIRQGT 294
              NFG  ++++ GT
Sbjct: 294   -NFGGGEMLKLGT 305
```

```
>gi|23295765|gb|AAL80036.1|          nucleocapsid     protein     [porcine
hemagglutinating encephalomyelitis virus]
           Length = 449

 Score =  145 bits (365), Expect = 1e-33
 Identities = 107/253 (42%), Positives = 145/253 (57%), Gaps = 18/253 (7%)

Query: 49  SWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGKMKELSPRW 106
            SWF+ +TQ  K +E  F  GQGVPI         +GY+ R  RR  +  DG  ++L PRW
Sbjct: 64  SWFSGITQFQKGKEFEFAEGQGVPIAPGVPSTEAKGYWYRHNRRSFKTADGNQRQLLPRW 123

Query: 107 YFYYLGTGPEASLPYGANKEGIVWVATEGA-LNTPKDHIGTRNPNNNAATVLQLPQGTTL 165
            YFYYLGTGP A   YG + +G+ WVA+  A +NTP D I  R+P+++ A   + P GT L
Sbjct: 124 YFYYLGTGPHAKDQYGTDIDGVFWVASNQADINTPAD-IVDRDPSSDEAIPTRFPPGTVL 182

Query: 166 PKGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRL 225
            P+G+Y EGS G S  +SRS+SR+  N   S    SR NS  R ++  G    +A    D++
Sbjct: 183 PQGYYIEGS-GRSAPNSRSTSRA-PNRAPSAGSRSRANSGNRTSTPGVTPDMA----DQI 236

Query: 226 NQLESKVSGKGQQQQGQTVTKKSAAEASK----KPRQKRTATKQYNVTQAFGRRGPEQTQ 281
              L     GK    + Q VTK++A E  +    KPRQKR+  KQ  V Q FG+RGP Q
Sbjct: 237 ASLVLAKLGK-DATKPQQVTKQTAKEVRQKILNKPRQKRSPNKQCTVQQCFGKRGPNQ-- 293

Query: 282 GNFGDQDLIRQGT 294
             NFG  ++++ GT
Sbjct: 294 -NFGGGEMLKLGT 305
```

These results indicate that SEQ ID NO: 10533 has functional similarities to a coronavirus nucleocapsid protein.

In one embodiment, the invention comprises an amino acid sequence from the 5'3' Frame 1 of Figure 127 *e.g.* SEQ ID NO[S]: 10506-10514. Some encoded open reading frames within this region are SEQ ID NO[S]: 10575 to 10578.

Accordingly, the invention includes a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 10575, SEQ ID NO: 10576, SEQ ID NO: 10577 and SEQ ID NO: 10578. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to a sequence selected from the group consisting of SEQ ID NO: 10097, SEQ ID NO: 10576, SEQ ID NO: 10577 and SEQ ID NO: 10578. The invention includes a fragment of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10097, SEQ ID NO: 10576, SEQ ID NO: 10577 and SEQ ID NO: 10578.

In one embodiment, the invention includes a polypeptide comprising an amino acid sequence from the 3'5' Frame 2 of Figure 127 *e.g.* SEQ ID NO[S]: 10547-10559. An open reading frame within this region is SEQ ID NO: 10579.

The invention includes a polypeptide comprising an amino acid sequence of SEQ ID NO: 10579. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 10579. The invention includes a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO: 10579.

The invention also includes polynucleotide sequences which can be used as probes for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified in Table 33. The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified in Table 33.

The invention includes a polynucleotide sequence comprising SEQ ID NO: 11323. A polypeptide encoded by SEQ ID NO: 11323 is SEQ ID NO: 11324.

The invention includes a polypeptide comprising SEQ ID NO: 11324, sequence having sequence identity to SEQ ID NO: 11324 and fragments of SEQ ID NO: 11324. The invention includes a fragment of SEQ ID NO: 11324, wherein said polypeptide fragment begins with a Methionine.

Accordingly, the invention includes a polynucleotide sequence comprising SEQ ID NO: 11323. It also provides polynucleotide sequences having sequence identity to SEQ ID NO: 11323. The invention also provides for polynucleotide sequences comprising fragments of SEQ ID NO: 11323. In one embodiment, the polynucleotide fragment does not consist entirely of a known SARS polynucleotide sequence or a known coronavirus polynucleotide sequence.

The invention includes an amino acid sequence encoded by the polynucleotide sequence SEQ ID NO: 11323, including the amino acid sequence of SEQ ID NO: 11324.

The invention also provides amino acid sequences having sequence identity to an amino acid sequence encoded by SEQ ID NO: 11323. The invention provides amino acid sequences having sequence identity to SEQ ID NO: 11324.

The invention provides fragments of amino acid sequences encoded by SEQ ID NO: 11323. The invention also provides fragments of amino acid sequences of SEQ ID NO: 11324. In one embodiment, the fragment does not consist entirely of a known SARS amino acid sequence or a known coronavirus amino acid sequence.

The invention also includes polynucleotide sequences which can be used as probes for diagnostic reagents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample. The invention includes a polynucleotide sequence comprising one or more of the primer sequences identified as SEQ ID NO^{S}: 11325-11440 (left part) and SEQ ID NO^{S}: 11441-11551 (right part). The invention further includes polynucleotide sequence comprising the complement of one or more of the primer sequences identified as SEQ ID NO^{S}: 11325-11551.

The invention includes a polypeptide comprising SEQ ID NO: 11552. The SARS virus contains polymorphism at the Isoleucine residue Ile-324. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11552, wherein

said polypeptide includes an amino acid sequence selected from the group consisting of YSYAI (SEQ ID NO: 11553), SYAIH (SEQ ID NO: 11554), YAIHH (SEQ ID NO: 11555), IHHDK (SEQ ID NO: 11556), SYAI (SEQ ID NO: 11557), YAIH (SEQ ID NO: 11558), AIHH (SEQ ID NO: 11559), IHHD (SEQ ID NO: 11560), YAI, AIH, and IHH. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11552, wherein said fragment includes an amino acid sequence selected from the group consisting of YSYAI (SEQ ID NO: 11553), SYAIH (SEQ ID NO: 11554), YAIHH (SEQ ID NO: 11555), IHHDK (SEQ ID NO: 11556), SYAI (SEQ ID NO: 11557), YAIH (SEQ ID NO: 11558), AIHH (SEQ ID NO: 11559), IHHD (SEQ ID NO: 11560), YAI, AIH, and IHH.

The invention includes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11561 and SEQ ID NO: 11562. The invention includes a fragment of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11561 and SEQ ID NO: 11562.

The invention includes a diagnostic kit comprising a polypeptide comprising at least one of the amino acid sequences selected from the group consisting of SEQ ID NO^S: 11561 and 11562. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding a polypeptide comprising at least one of the amino acid sequences selected from the group consisting of SEQ ID NO^S: 11561 and 11562. The invention includes an immunogenic composition comprising a polypeptide comprising at least one of the amino acid sequences selected from the group consisting of SEQ ID NO^S: 11561 and 11562. The invention includes an antibody which recognizes a polypeptide comprising at least one of the amino acid sequences selected from the group consisting of SEQ ID NO^S: 11561 and 11562.

The invention includes a polynucleotide sequence SEQ ID NO: 11563 or a fragment thereof or a sequence having sequence identity thereto. Polypeptide sequences which can be translated from SEQ ID NO: 11563 are shown in Figure 128. The constituent amino acid sequences from Figure 128, having at least 4 amino acids, are listed as SEQ ID NO^S: 11564 to 11617.

The invention includes a polypeptide sequence selected from the group consisting of the sequences of Figure 128, or a fragment thereof or a sequence having sequence identity thereto *e.g.* SEQ ID NO^S: 11563 to 11617.

A polypeptide sequence within SEQ ID NO: 11600 is SEQ ID NO: 11618. The invention includes a polypeptide comprising SEQ ID NO: 11618, or a fragment thereof or a sequence having sequence identity thereto.

A polypeptide sequence within SEQ ID NO: 11602 is SEQ ID NO: 11641. The invention includes a polypeptide comprising SEQ ID NO: 11641, or a fragment thereof or a sequence having sequence identity thereto.

A polypeptide sequence within SEQ ID NO: 11609 is SEQ ID NO: 11619.

The invention includes a polynucleotide encoding (i) an amino acid sequence selected from the group consisting of: (1) the amino acid sequences of Figure 128, and in particular SEQ ID NO^{S}: 11564-11617; (2) SEQ ID NO: 11618; and (3) SEQ ID NO: 11619, or (ii) a fragment thereof. The invention includes a diagnostic kit comprising a one or more of these proteins. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding one or more of these polypeptide sequences. The invention includes an antibody which recognizes one or more of the polypeptide sequences.

The SARS virus may contain polymorphism at isoleucine residue Ile-326 in SEQ ID NO: 11620 (Chi-PEP3). The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11620, wherein said polypeptide includes an amino acid sequence selected from the group consisting of YAIHH (SEQ ID NO: 11621) and YATHH (SEQ ID NO: 11622). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11620, wherein said fragment includes an amino acid sequence selected from the group consisting of YAIHH (SEQ ID NO: 11621) and YATHH (SEQ ID NO: 11622).

The SARS virus may contain polymorphism at glutamine residue Gln-830 in SEQ ID NO: 11620. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11620, wherein said polypeptide includes an amino acid sequence selected from the group consisting of ASQAW (SEQ ID NO: 11623) and ASRAW (SEQ ID NO: 11624). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11620, wherein said fragment includes an amino acid sequence selected from the group consisting of ASQAW (SEQ ID NO: 11623) and ASRAW (SEQ ID NO: 11624).

The SARS virus may contain polymorphism at aspartic acid residue Asp-935 in SEQ ID NO: 11620. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11620, wherein said polypeptide includes an amino acid sequence selected from the group consisting of DADST (SEQ ID NO: 11625) and DAYST (SEQ ID NO: 11626). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11620, wherein said fragment includes an amino acid sequence selected from the group consisting of DADST (SEQ ID NO: 11625) and DAYST (SEQ ID NO: 11626).

The SARS virus may contain polymorphism at serine residue Ser-577 in SEQ ID NO: 11627 (Chi-PEP4). The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11627, wherein said polypeptide includes an amino

acid sequence selected from the group consisting of PCSFG (SEQ ID NO: 11628) and PCAFG (SEQ ID NO: 11629). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11627, wherein said fragment includes an amino acid sequence selected from the group consisting of PCSFG (SEQ ID NO: 11628) and PCAFG (SEQ ID NO: 11629).

The SARS virus may contain polymorphism at valine residue Val-68 in SEQ ID NO: 11630 (Chi-PEP8). The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11630, wherein said polypeptide includes an amino acid sequence selected from the group consisting of LAVVY (SEQ ID NO: 11631) and LAAVY (SEQ ID NO: 11632). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11630, wherein said fragment includes an amino acid sequence selected from the group consisting of LAVVY (SEQ ID NO: 11631) and LAAVY (SEQ ID NO: 11632).

The SARS virus may contain polymorphism at isoleucine residue Ile-50 in SEQ ID NO: 11633 (Chi-PEP13). The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11633, wherein said polypeptide includes an amino acid sequence selected from the group consisting of NNIAS (SEQ ID NO: 11634) and NNTAS (SEQ ID NO: 11635). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11633, wherein said fragment includes an amino acid sequence selected from the group consisting of NNIAS (SEQ ID NO: 11634) and NNTAS (SEQ ID NO: 11635).

The SARS virus may contain a polymorphism at Serine residue Ser-943 in SEQ ID NO: 11636. The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 11636, wherein said polypeptide includes an amino acid sequence selected from the group consisting of AVSAC (SEQ ID NO: 11637) and AVGAC (SEQ ID NO: 11638). The invention includes a fragment of a polypeptide comprising SEQ ID NO: 11636, wherein said fragment includes an amino acid seuence selected from the group consisting of AVSAC (SEQ ID NO: 11637) and AVGAC (SEQ ID NO: 11638).

The invention includes a polynucleotide SEQ ID NO: 11639, or a fragment thereof or a sequence having sequence identity thereto. The invention includes a polypeptide encoded by the polynucleotide sequence set forth in SEQ ID NO: 11639, or a fragment thereof or a polypeptide sequence having sequence identity thereto.

The invention includes a polynucleotide set forth in SEQ ID NO: 11640, or a fragment thereof or a sequence having sequence identity thereto. The invention includes a polypeptide encoded by the polynucleotide sequence set forth in SEQ ID NO: 11640, or a fragment thereof or a polypeptide sequence having sequence identity thereto.

The invention includes each of the polynucleotides identified above. The invention includes each of the polynucleotides set forth in the sequence listing. The invention further

includes polynucleotides having sequence identity to each of the polynucleotides identified above. The degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

The invention includes polynucleotide sequences comprising fragments of each of the polynucleotide sequences identified above. The fragments should comprise at least *n* consecutive polynucleotides from a particular SEQ ID NO:, and, depending on the sequence, *n* is 7 or more (*e.g.*, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more).

The invention includes each of the amino acid sequences encoded by each of the polynucleotide sequences identified above. The invention includes each of the amino acid sequences encoded by each of the polynucleotide sequences set forth in the sequence listing. The invention further includes amino acid sequences having sequence identity to the amino acid sequences encoded by each of the polynucleotide sequences identified above. The degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more). The invention further includes fragments of amino acid sequences encoded by each of the polynucleotide sequences identified above. The fragments should comprise at least *n* consecutive amino acids from a particular SEQ ID NO:, and, depending on the sequence, *n* is 7 or more (*e.g.*, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more).

The invention includes each of the amino acid sequences identified above. The invention includes each of the amino acid sequence set forth in the sequence listing. The invention further includes amino acid sequences having sequence identity to each of the amino acid sequences identified above. The degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

The invention further includes fragments of the amino acid sequences identified above. The fragments should comprise at least *n* consecutive amino acids from a particular SEQ ID NO:, and, depending on the sequence, *n* is 7 or more (*e.g.*, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55,

60, 65, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more).

The invention includes polynucleotides encoding each of the amino acid sequences identified above. The invention includes polynucleotides encoding each of the amino acid sequences set forth in the sequence listing. The invention further includes polynucleotides having sequence identity with each of the polynucleotides encoding each of the amino acid sequences identified above. The degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

The invention further includes fragments of polynucleotides encoding each of the amino acid sequences identified above. The fragments should comprise at least *n* consecutive polynucleotides from a particular SEQ ID NO:, and, depending on the sequence, *n* is 7 or more (*e.g.*, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more).

As described in more detail below, polynucleotides for use as primers and/or as probes may contain at least 4 or 8 contiguous nucleotides from a polynucleotide sequence of the invention *e.g.* at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides and up to about 50, 75, 100, 200 contiguous nucleotides or more. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and about 13, 14, 15, 16, 17, 18, 19, 20, or 21 or more nucleotides or more appears optimal for hybridisation.

In one embodiment, the invention is directed to polynucleotides and amino acid sequences that do not consist entirely of a known SARS virus polynucleotide or amino acid sequence or of a known coronavirus polynucleotide or amino acid sequence. In one embodiment, the polynucleotides and amino acid sequences of the invention do not consist entirely of the sequence SEQ ID NO: 1. In another embodiment, the polynucleotides and amino acid sequences of the invention do not consist entirely of the sequence SEQ ID NO: 2. SEQ ID NO: 9967 is a SARS genome sequence of the Frankfurt (FRA) isolate (GenBank: AY310120). Compared to SEQ ID NO: 1, it differs at nucleotides 2546, 2590, 11437, 18954, 19073, 20585, 20899, 23209, 24922, 26589 & 28257; compared to SEQ ID NO:2, it differs at nucleotides 2560, 7922, 11451, 16625, 18968 & 19067. Further genome sequences have become available from GenBank, since this application was originally filed, under accession numbers including AY559097, AY559096, AY559095, AY559094, AY559093, AY559092, AY559091, AY559090, AY559089, AY559088, AY559087, AY559086, AY559085, AY559084, AY559083, AY559082, AY559081, AY274119,

AY323977, AY291315, AY502932, AY502931, AY502930, AY502929, AY502928, AY502927, AY502926, AY502925, AY502924, AY502923, AY291451, AY390556, AY395003, AY395002, AY395001, AY395000, AY394999, AY394998, AY394997, AY394996, AY394995, AY394994, AY394993, AY394992, AY394991, AY394990, AY394989, AY394987, AY394986, AY394985, AY394983, AY394979, AY394978, AY508724, AY394850, AY463059, AY463060, AY313906, AY310120, AY461660, AY485278, AY485277, AY345988, AY345987, AY345986, AY282752, AY357076, AY357075, AY350750, AY304495, AY304488, AY304486, AY427439, AY283798, AY278491, AY278489, AY362699, AY362698, AY283797, AY283796, AY283795, AY283794, AY278741, AY351680, AP006561, AP006560, AP006559, AP006558, AP006557, AY278554, AY348314, AY338175, AY338174, AY321118, AY279354, AY278490, AY278487, AY297028, AY278488, and NC_004718.

In another embodiment, the invention is directed to polynucleotides that encode proteins which are not immunologically cross reactive with a protein of a mouse hepatitis virus, a bovine coronavirus or an avian infectious bronchitis virus. In another embodiment, the invention is directed to proteins which are not immunologically cross reactive with a protein of a mouse hepatitis virus, a bovine coronavirus or an avian infectious bronchitis virus.

Each of the polynucleotides identified above may be used to encode a portion of a fusion protein. Accordingly, the invention compries one or more of the polynucleotides identified above wherein the polynucleotides encoding for the start codon are removed. The invention further comprises one or more of the amino acids identified above wherein the starting methionine is removed.

Any of the polynucleotide or amino acid sequences discussed above may be used in vaccines for the treatment or prevention of SARS virus infection, including as a SARS viral antigen. Additionally, any of the polynucleotides or amino acid sequences discussed above may be used as diagnostic reagents, or in kits (comprising such reagents) or in methods used to diagnose or identify the presence or absence of a SARS virus in a biological sample.

SARS viral antigens of the invention may include a polypeptide with 99%, 95%, 90%, 85%, or 80% homology to one or more of the group consisting of the following proteins: nonstructural protein 2 (NS2); hemagglutinin-esterase glycoprotein (HE) (also referred to as E3), spike glycoprotein (S) (also referred to as E2), nonstructural region 4 (NS4), envelope (small membrane) protein (E) (also referred to as sM), membrane glycoprotein (M) (also referred to as E1), nucleocapsid phosphoprotein (N) or RNA dependent RNA polymerase (pol).

A detailed discussion of Coroavirus biology can be found in *Fields Virology* (2nd ed), Fields *et al.* (eds.), B.N. Raven Press, New York, NY., Chapter 35.

Another example of a SARS virus isolate is set forth in Example 1 below. The invention includes each of the polypeptide and polynucleotide sequences identified in Example 1. In

addition, the invention includes vaccine formulations comprising one or more of the polypeptide or polynucleotide sequences identified in Example 1. The invention includes diagnostic regaents, kits (comprising such reagents) and methods which can be used to diagnose or identify the presence or absence of a SARS virus in a biological sample using one or more of the polypeptide or polynucleotide sequences identified in Example 1. The invention includes methods for the treatment or prevention of SARS virus infection utilizing small molecule viral inhibitors and combinations of small molecule viral inhibitors and kits for the treatment of SARS. The small molecule inhibitors may specifically target one or more of the polypeptides or polynucleotides identified in Example 1.

Further discussion of terms used in the application follows below.

"Respiratory Virus" as used herein refers to a virus capable of infecting the human respiratory tract. Respiratory Viral Antigens suitable for use in the invention include Severe Acute Respiratory Syndrome virus, coronavirus, influenza virus, human rhinovirus (HRV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, and rhinovirus.

The terms "polypeptide", "protein" and "amino acid sequence" as used herein generally refer to a polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, mulimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. Minimum fragments of polypeptides useful in the invention can be at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or even 15 amino acids. Typically, polypeptides useful in this invention can have a maximum length suitable for the intended application. Generally, the maximum length is not critical and can easily be selected by one skilled in the art.

Polypeptides of the invention can be prepared in many ways *e.g.* by chemical synthesis (at least in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (*e.g.* from recombinant expression), from the organism itself (*e.g.* after viral culture, or direct from patients), from a cell line source *etc.* A preferred method for production of peptides <40 amino acids long involves *in vitro* chemical synthesis (Bodanszky (1993) *Principles of Peptide Synthesis* (ISBN: 0387564314); Fields *et al.* (1997) *Methods in Enzymology 289: Solid-Phase Peptide Synthesis*. ISBN: 0121821900). Solid-phase peptide synthesis is particularly preferred, such as methods based on t-Boc or Fmoc (Chan & White (2000) *Fmoc Solid Phase Peptide Synthesis* ISBN: 0199637245) chemistry. Enzymatic synthesis (Kullmann (1987) *Enzymatic Peptide Synthesis*. ISBN: 0849368413) may also be used in part or in full. As an alternative to chemical synthesis, biological synthesis may be used *e.g.* the polypeptides may be produced by translation. This may be carried out in vitro or in vivo. Biological methods are in general restricted to the production of polypeptides based on L-amino

acids, but manipulation of translation machinery (*e.g.* of aminoacyl tRNA molecules) can be used to allow the introduction of D-amino acids (or of other non natural amino acids, such as iodotyrosine or methylphenylalanine, azidohomoalanine, *etc.*) (Ibba (1996) *Biotechnol Genet Eng Rev* 13:197-216.). Where D-amino acids are included, however, it is preferred to use chemical synthesis. Polypeptides of the invention may have covalent modifications at the C-terminus and/or N-terminus, particularly where they are for *in vivo* administration *e.g* by attachment of acetyl or carboxamide, as in the Fuzeon™ product.

Reference to polypeptides and the like also includes derivatives of the amino acid sequences of the invention. Such derivatives can include postexpression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation, and the like. Amino acid derivatives can also include modifications to the native sequence, such as deletions, additions and substitutions (generally conservative in nature), so long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification. Furthermore, modifications may be made that have one or more of the following effects: reducing toxicity; facilitating cell processing (*e.g.*, secretion, antigen presentation, *etc.*); and facilitating presentation to B-cells and/or T-cells.

"Fragment" or "Portion" as used herein refers to a polypeptide consisting of only a part of the intact full-length polypeptide sequence and structure as found in nature. For instance, a fragment can include a C-terminal deletion and/or an N-terminal deletion of a protein.

A "recombinant" protein is a protein which has been prepared by recombinant DNA techniques as described herein. In general, the gene of interest is cloned and then expressed in transformed organisms, as described further below. The host organism expressed the foreign gene to produce the protein under expression conditions.

The term "polynucleotide", as known in the art, generally refers to a nucleic acid molecule. A "polynucleotide" can include both double- and single-stranded sequences and refers to, but is not limited to, cDNA from viral, prokaryotic or eukaryotic mRNA, genomic RNA and DNA sequences from viral (*e.g.* RNA and DNA viruses and retroviruses) or prokaryotic DNA, and especially synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA, and includes modifications such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the nucleic acid molecule encodes a therapeutic or antigenic protein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts that produce the antigens. Modifications of polynucleotides may have any number of effects including, for example, facilitating expression of the polypeptide product in a host cell.

Polynucleotides of the invention may be prepared in many ways *e.g.* by chemical synthesis (*e.g.* phosphoramidite synthesis of DNA) in whole or in part, by digesting longer nucleic acids using nucleases (*e.g.* restriction enzymes), by joining shorter nucleic acids or nucleotides (*e.g.* using ligases or polymerases), from genomic or cDNA libraries, *etc.*

A polynucleotide can encode a biologically active (*e.g.*, immunogenic or therapeutic) protein or polypeptide. Depending on the nature of the polypeptide encoded by the polynucleotide, a polynucleotide can include as little as 10 nucleotides, *e.g.*, where the polynucleotide encodes an antigen.

By "isolated" is meant, when referring to a polynucleotide or a polypeptide, that the indicated molecule is separate and discrete from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose. The polynucleotides and polypeptides of the invention are preferably isolated polynucleotides and isolated polypeptides.

"Antibody" as known in the art includes one or more biological moieties that, through chemical or physical means, can bind to or associate with an epitope of a polypeptide of interest. The antibodies of the invention include antibodies which specifically bind to a SARS viral antigen. The term "antibody" includes antibodies obtained from both polyclonal and monoclonal preparations, as well as the following: hybrid (chimeric) antibody molecules (see, for example, Winter *et al.* (1991) *Nature* 349: 293-299; and US Patent No. 4,816,567; $F(ab')_2$ and F(ab) fragments; F_v molecules (non-covalent heterodimers, see, for example, Inbar *et al.* (1972) *Proc Natl Acad Sci USA* 69:2659-2662; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096); single-chain Fv molecules (sFv) (see, for example, Huston *et al.* (1988) *Proc Natl Acad Sci USA* 85:5897-5883); dimeric and trimeric antibody fragment constructs; minibodies (see, *e.g.*, Pack *et al.* (1992) *Biochem* 31:1579-1584; Cumber *et al.* (1992) *J Immunology* 149B: 120-126); humanized antibody molecules (see, for example, Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyan *et al.* (1988) *Science* 239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994); and, any functional fragments obtained from such molecules, wherein such fragments retain immunological binding properties of the parent antibody molecule. The term "antibody" further includes antibodies obtained through non-conventional processes, such as phage display.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. Thus, the term encompasses antibodies obtained from murine hybridomas, as well as human monoclonal

antibodies obtained using human rather than murine hybridomas. See, *e.g.*, Cote, *et al.* *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, 1985, p 77.

An "immunogenic composition" as used herein refers to a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest. The immunogenic composition can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal or any other parenteral, mucosal or transdermal (*e.g.*, intra-rectally or intra-vaginally) route of administration.

The term "derived from" is used to identify the source of molecule (*e.g.*, a molecule can be derived from a polynucleotide, polypeptide, an immortalized cell line can be derived from any tissue, *etc.*). A first polynucleotide is "derived from" a second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above. Thus, a first polynucleotide sequence is "derived from" a second sequence if it has (i) the same or substantially the same sequence as the second sequence or (ii) displays sequence identity to polypeptides of that sequence.

A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above. Thus, a polypeptide (protein) is "derived from" a particular SARS virus if it is (i) encoded by an open reading frame of a polynucleotide of that SARS virus, or (ii) displays sequence identity, as described above, to polypeptides of that SARS virus.

Both polynucleotide and polypeptide molecules can be physically derived from a SARS virus or produced recombinantly or synthetically, for example, based on known sequences.

A cultured cell or cell line is "derived from" another cell, cells or tissue if it is originally obtained from existing cells or tissue. Non-limiting examples of tissue that cells may be derived from include skin, retina, liver, kidney, heart, brain, muscle, intestinal, ovary, breast, prostate, cancerous tissue, tissue infected with one or more pathogens (*e.g.*, viruses, bacteria *etc.*) and the like. The cells described herein may also be derived from other cells including, but not limited to, primary cultures, existing immortalized cells line and/or other isolated cells.

An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen." Normally, an epitope will include between about 3-15, generally about 5-15 amino acids. A B-cell epitope is normally about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a

helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (*i.e.*, antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes as well as tumor antigens, including extracellular domains of cell surface receptors and intracellular portions that may contain T-cell epitopes. Antibodies such as anti-idiotype antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide that expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to an immune response mediated by antibody molecules, including secretory (IgA) or IgG molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells. In addition, a chemokine response may be induced by various white blood or endothelial cells in response to an administered antigen.

II. VACCINE FORMULATIONS

The invention relates to vaccine formulations for the treatment or prevention of Severe Acute Respiratory Syndrome (SARS). Vaccine formulations of the invention include an inactivated (or killed) SARS virus, an attenuated SARS virus, a split SARS virus preparation and a recombinant or purified subunit formulation of one or more SARS viral antigens. The invention includes polypeptides and polynucleotides encoding for SARS viral antigens and

immunogenic fragments thereof. Expression and delivery of the polynucleotides of the invention may be facilitated via viral vectors and/or viral particles, including Virus Like Particles (VLPs).

A. Inactivated (or Killed) SARS Vaccines

The invention includes a composition comprising an inactivated (or killed) SARS virus and methods for the production thereof. Inactivated SARS viral compositions can be used as prophylactic or therapeutic SARS virus vaccine. Preferably the inactivated SARS virus vaccine composition comprises an amount of inactivated SARS virus which, before inactivation, is equivalent to a virus titer of from about 4 to 7 logs plaque forming units (PFU) or 4 to 7 logs tissue culture infectious dose 50 ($TCID_{50}$) per milliliter. More preferably, before inactivation the virus titer is from 4 to 11, 7 to 11 or 9 to 11 PFU or $TCID_{50}$. Still more preferably the inactivated SARS virus vaccine composition comprises an amount of inactivated SARS virus which, before inactivation, is equivalent to a virus titer of from about 5 to 9 PFU or 5 to 9 $TCID_{50}$ per milliliter. In one embodiment, the PFU or $TCID_{50}$ of the cultured SARS virus at harvest is 6 to 8, more preferably about 7.5 PFU or $TCID_{50}$ per milliliter. Upon concentration of the viral harvest, the PFU or $TCID_{50}$ is preferably 8 to 11, still more preferably about 9 PFU or $TCID_{50}$ per milliliter. The vaccine composition comprises a sufficient amount of the SARS virus antigen to produce an immunological response in a primate.

Methods of inactivating or killing viruses are known in the art to destroy the ability of the viruses to infect mammalian cells. Such methods include both chemical or physical means. Chemical means for inactivating a SARS virus include treatment of the virus with an effective amount of one or more of the following agents: detergents, formaldehyde, formalin, β-propiolactone, or UV light. Additional chemical means for inactivation include treatment with methylene blue, psoralen, carboxyfullerene (C60) or a combination of any thereof. Other methods of viral inactivation are known in the art, such as for example binary ethylamine, acetyl ethyleneimine, or gamma irradiation.

For example formaldehyde may be used at concentrations such as 0.1 to 0.02%, preferably at 0.02 to 0.1 %, and still more preferably at 0.04 to 0.05%. The inactivating agent is added to virus containing culture supernatants prior to or after harvesting said culture supernatants from vessels used for virus propagation, either with or without a step of cell disruption for release of cell-associated virus prior to harvesting. Further, the inactivating agent may be added after said culture supernatants have been stored frozen and thawed, or after one or more steps of purification to remove cell contaminants. Preferably, however, formaldehyde is added after removal of cells and cellular debris or after one or more purification steps. After addition of formaldehyde, the virus containing mixture is transferred into an incubation vessel and incubated at refrigeration temperatures (e.g. +2 to 8°C) or alternatively at elevated temperatures, such as ambient temperatures between approximately 20 and 30°C or at 33°C to 37°C for a period of 12

hours to 7 days, whereby the temperature chosen should be adjusted to the duration of incubation. Prefered conditions are e.g. +2 –8°C for 3-7 days (prefered are 3 -7days), ambient temperatures and incubation for 16 hours to 3 days (prefered 24- 48 hours), or 35-37°C for 12-36 hours. If it is desirable to remove excess formalin, sodium thiosulfate or sodium metabisulfite at equimolar or 1.5 -fold molar concentration (relative to formaldehyde) may be added after completing the inactivation process.

For example, β-propiolactone may be used at concentrations such as 0.01 to 0.5%, preferably at 0.5% to 0.2%, and still more preferably at 0.025 to 0.1%. The inactivating agent is added to virus containing culture supernatants (virus material) prior to or after harvesting said culture supernatants from vessels used for virus propagation, either with or without a step of cell disruption for release of cell-associated virus prior to harvesting. Further, the inactivating agent may be added after said culture supernatants have been stored frozen and thawed, or after one or more steps of purification to remove cell contaminants. β-propiolactone is added to the virus material, with the adverse shift in pH to acidity being controlled with sodium hydroxide (*e.g.*, 1 N NaOH), a Tris-buffer or sodium bicarbonate solution. After transfering the mixture to another inactivation vessel, the combined inactivating agent-virus materials are incubated at temperatures from 4°C to 37°C, for incubation times of preferably 24 to 72 hours.

Another inactivant which may be used is binary ethyleneimine (BEI). Equal volumes of a 0.2 molar bromoethylamine hydrobromide solution and a 0.4 molar sodium hydroxide solution are mixed and incubated at about 37°C. for 60 minutes. The resulting cyclized inactivant is binary ethyleneimine, which is added to the virus materials at 0.5 to 4 percent, and preferably at 1 to 3 percent, volume to volume. The inactivating virus materials are held from about 4°C to 37°C for 24 to 72 hours with periodic agitation. At the end of this incubation 20 ml. of a sterile 1 molar sodium thiosulfate solution was added to insure neutralization of the BEI.

In one embodiment, the invention includes an inactivating method is designed to maximize exposure of the virus to the inactivating agent and to minimize long-term exposure of the temperature sensitive SARS virus particles to elevated temperatures. The invention includes an inactivation method comprising exposing the virus to the inactivation agent (such as BPL) for 12 to 24 hours at refrigeration temperatures followed by hydrolysis of any residual inactivating agent by elevating the temperature for only 3 hours. Preferably, the refrigeration temperatures are between 0 and 8°C, more preferably around 4°C. Preferably, the elevated temperature is between 33 and 41°C, more preferably around 37°C. As assessed by a test for residual infectious virus using 10 ml aliquots of the inactivated preparation, the method is able to inactivate SARS-CoV in raw cell culture harvests below a theoretical limit of 0.03 infectious units/ml.

Diluted and undiluted samples of the inactivated virus materials are added to susceptible cell (tissue) culture (*e.g.*, VERO) to detect any non-inactivated virus. The cultured cells are

passaged multiple times and examined for the presence of SARS virus based on any of a variety of methods, such as, for example, cytopathic effect (CPE) and antigen detection (*e.g.*, via fluoroscent antibody conjugates specific for SARS virus). Such tests allow determination of complete virus inactivation.

Prior to inactivation, the SARS virus will be cultured in a mammalian cell culture. The cell culture may be adherently growing cells or cells growing in suspension. Preferably the cells are of mammalian origin, but may also be derived from avian (*e.g.*, hens' cells such as hens' embryo cells (CEF cells)), amphibian, reptile, insect, or fish sources. Mammalian sources of cells include, but are not limited to, human or non-human primate (*e.g.*, MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75), HeLa cells, human diploid cells, fetal rhesus lung cells (*e.g.* ATCC CL-160), human embryonic kidney cells (293 cells, typically transformed by sheared adenovirus type 5 DNA), VERO cells (*e.g.*, from monkey kidneys), horse, cow (*e.g.*, MDBK cells), sheep, dog (*e.g.*, MDCK cells from dog kidneys, ATCC CCL34 MDCK (NBL2) or MDCK 33016, deposit number DSM ACC 2219 as described in WO 97/37001), cat, and rodent (*e.g.*, hamster cells such as BHK21-F, HKCC cells, or Chinese hamster ovary cells (CHO cells)), and may be obtained from a wide variety of developmental stages, including for example, adult, neonatal, fetal, and embryo.

In certain embodiments the cells are immortalized (*e.g.*, PERC.6 cells are described, for example, in WO 01/38362 and WO 02/40665, incorporated by reference herein in their entireties, as well as deposited under ECACC deposit number 96022940), or any other cell type immortalized using the techniques described herein.

In preferred embodiments, mammalian cells are utilized, and may be selected from and/or derived from one or more of the following non-limiting cell types: fibroblast cells (*e.g.*, dermal, lung), endothelial cells (*e.g.*, aortic, coronary, pulmonary, vascular, dermal microvascular, umbilical), hepatocytes, keratinocytes, immune cells (*e.g.*, T cell, B cell, macrophage, NK, dendritic), mammary cells (*e.g.*, epithelial), smooth muscle cells (*e.g.*, vascular, aortic, coronary, arterial, uterine, bronchial, cervical, retinal pericytes), melanocytes, neural cells (*e.g.*, astrocytes), prostate cells (*e.g.*, epithelial, smooth muscle), renal cells (*e.g.*, epithelial, mesangial, proximal tubule), skeletal cells (*e.g.*, chondrocyte, osteoclast, osteoblast), muscle cells (*e.g.*, myoblast, skeletal, smooth, bronchial), liver cells, retinoblasts, and stromal cells. WO 97/37000 and WO 97/37001, incorporated by reference herein in their entireties, describe production of animal cells and cell lines that capable of growth in suspension and in serum free media and are useful in the production and replication of viruses.

Preferably, the SARS viruses of the invention are grown on VERO cells or fetal rhesus kidney cells.

Culture conditions for the above cell types are well-described in a variety of publications, or alternatively culture medium, supplements, and conditions may be purchased commercially, such as for example, as described in the catalog and additional literature of Cambrex Bioproducts (East Rutherford, NJ).

In certain embodiments, the host cells used in the methods described herein are cultured in serum free and/or protein free media. A medium is referred to as a serum-free medium in the context of the present invention in which there are no additives from serum of human or animal origin. Protein-free is understood to mean cultures in which multiplication of the cells occurs with exclusion of proteins, growth factors, other protein additives and non-serum proteins. The cells growing in such cultures naturally contain proteins themselves.

Known serum-free media include Iscove's medium, Ultra-CHO medium (BioWhittaker) or EX-CELL (JRH Bioscience). Ordinary serum-containing media include Eagle's Basal Medium (BME) or Minimum Essential Medium (MEM) (Eagle, Science, 130, 432 (1959)) or Dulbecco's Modified Eagle Medium (DMEM or EDM), which are ordinarily used with up to 10% fetal calf serum or similar additives. Optionally, Minimum Essential Medium (MEM) (Eagle, Science, 130, 432 (1959)) or Dulbecco's Modified Eagle Medium (DMEM or EDM) may be used without any serum containing supplement. Protein-free media like PF-CHO (JHR Bioscience), chemically-defined media like ProCHO 4CDM (BioWhittaker) or SMIF 7 (Gibco/BRL Life Technologies) and mitogenic peptides like Primactone, Pepticase or HyPep™ (all from Quest International) or lactalbumin hydrolyzate (Gibco and other manufacturers) are also adequately known in the prior art. The media additives based on plant hydrolyzates have the special advantage that contamination with viruses, mycoplasma or unknown infectious agents can be ruled out.

The cell culture conditions to be used for the desired application (temperature, cell density, pH value, *etc.*) are variable over a very wide range owing to the suitability of the cell line employed according to the invention and can be adapted to the requirements of the SARS virus.

The method for propagating the SARS virus in cultured cells (*e.g.*, mammalian cells) includes the steps of inoculating the cultured cells with SARS virus, cultivating the infected cells for a desired time period for virus propagation, such as for example as determined by SARS virus titer or SARS virus antigen expression (*e.g.*, between 24 and 168 hours after inoculation) and collecting the propagated virus. The cultured cells are inoculated with a SARS virus (measured by PFU or $TCID_{50}$) to cell ratio of 1:10000 to 1:10. A lower range of ratios may also be used *e.g.* 1:500 to 1:1, preferably 1:100 to 1:5, more preferably 1:50 to 1:10. The SARS virus is added to a suspension of the cells or is applied to a monolayer of the cells, and the virus is absorbed on the cells for at least 60 minutes but usually less than 300 minutes, preferably between 90 and 240 minutes at 25°C to 40°C, more preferably 28°C to 37°C, still more

preferably at about 33 °C. The infected cell culture (*e.g.*, monolayers) may be treated either by freeze-thawing or by enzymatic action to increase the viral content of the harvested culture supernatants. The harvested fluids are then either inactivated or stored frozen.

A comparison of SARS infected Vero cells grown with and without fetal calf serum ("FCS") is shown in FIGURE 26A. Briefly, Vero cells were split the day before infection and cultivated in T175 flasks. Infection of 90% confluent Vero cell monolayers the following day was performed with a SARS-CoV seed stock (strain FRA, passage 4, Accession number AY310120), with or without 3% FCS (Fig. 26A). The addition of FCS to the cell media showed little impact on virus yield.

Cultured cells may be infected at a multiplicity of infection ("m.o.i.") of about 0.0001 to 10, preferably 0.002 to 5, more preferably to 0.001 to 2. Still more preferably, the cells are infected at a m.o.i of about 0.01. A comparison of viral yield at varying m.o.i. levels is shown in FIGURE 26B.

Infected cells may be harvested 30 to 60 hours post infection. Preferably, the cells are harvested 34-48 hours post infection. Still more preferably, the cells are harvested 38 to 40 hours post infection. See FIGURE 26C.

Methods of purification of inactivated virus are known in the art and may include one or more of, for instance gradient centrifugation, ultracentrifugation, continuous-flow ultracentrifugation and chromatography, such as ion exchange chromatography, size exclusion chromatography, and liquid affinity chromatography. Additional method of purification include ultrafiltration and dialfiltration. *See* JP Gregersen "Herstellung von Virussimpfstoffen aus Zellkulturen" Chapter 4.2 in Pharmazeutische Biotecnologie (eds. O. Kayser and RH Mueller). Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2000. *See also*, O'Neil *et al.*, "Virus Harvesting and Affinity Based Liquid Chromatography. A Method for Virus Concentration and Purification", Biotechnology (1993) 11:173-177; Prior *et al.*, "Process Development for Manufacture of Inactivated HIV-1", Pharmaceutical Technology (1995) 30-52; and Majhdi *et al.*, "Isolation and Characterization of a Coronavirus from Elk Calves with diarrhea" Journal of Clinical Microbiology (1995) 35(11): 2937-2942.

Other examples of purification methods suitable for use in the invention include polyethylene glycol or ammonium sulface precipitation (*see* Trepanier *et al.*, "Concentration of human respiratory syncytial virus using ammonium sulfate, polyethylene glycol or hollow fiber ultrafiltration" Journal of Virological Methods (1981) 3(4):201-211; Hagen *et al.*, "Optimization of Poly(ethylene glycol) Precipitation of Hepatitis Virus Used to prepare VAQTA, a Highly Purified Inactivated Vaccine" Biotechnology Progress (1996) 12:406-412; and Carlsson *et al.*, "Purification of Infectious Pancreatic Necrosis Virus by Anion Exchange Chromatography Increases the Specific Infectivity" Journal of Virological Methods (1994) 47:27-36) as well as

ultrafiltration and microfiltration (*see* Pay *et al.*, Developments in Biological Standardization (1985) 60:171-174; Tsurumi *et al.*, "Structure and filtration performances of improved cuprammonium regenerated cellulose hollow fibre (improved BMM hollow fibre) for virus removal" Polymer Journal (1990) 22(12):1085-1100; and Makino *et al.*, "Concentration of live retrovirus with a regenerated cellulose hollow fibre, BMM", Archives of Virology (1994) 139(1-2):87-96.).

Preferably, the virus is purified using chromatography, such as ion exchange chromatography. Chromatic purification allows for the production of large volumes of virus containing suspension. The viral product of interest can interact with the chromatic medium by a simple adsorption/desorption mechanism, and large volumes of sample can be processed in a single load. Contaminants which do not have affinity for the adsorbent pass through the column. The virus material can then be eluted in concentrated form.

Preferred anion exchange resins for use in the invention include DEAE, EMD TMAE. Preferred cation exchange resins may comprise a sulfonic acid-modified surface. In one embodiment, the virus is purified using ion exchange chromatography comprising a strong anion exchange resin (*e.g.* EMD TMAE) for the first step and EMD-SO_3 (cation exchange resin) for the second step. A metal-binding affinity chromatography step can optionally be included for further purification. (See, *e.g.*, WO 97/06243).

A preferred resin for use in the invention is Fractogel™ EMD. This synthetic methacrylate based resin has long, linear polymer chains (so-called "tentacles") covalently attached. This "tentacle chemistry" allows for a large amount of sterically accessible ligands for the binding of biomolecules without any steric hindrance. This resin also has improved pressure stability.

Column-based liquid affinity chromatography is another preferred purification method for use in the invention. One example of a resin for use in this purification method is Matrex™ Cellufine™ Sulfate (MCS). MCS consists of a rigid spherical (approx. 45-105 μm diameter) cellulose matrix of 3,000 Dalton exclusion limit (its pore structure excludes macromolecules), with a low concentration of sulfate ester functionality on the 6-position of cellulose. As the functional ligand (sulfate ester) is relatively highly dispersed, it presents insufficient cationic charge density to allow for most soluble proteins to adsorb onto the bead surface. Therefore the bulk of the protein found in typical virus pools (cell culture supernatants, *e.g.* pyrogens and most contaminating proteins, as well as nucleic acids and endotoxins) are washed from the column and a degree of purification of the bound virus is achieved.

The rigid, high-strength beads of MCS tend to resist compression. The pressure/flow characteristics the MCS resin permit high linear flow rates allowing high-speed processing, even in large columns, making it an easily scalable unit operation. In addition a chromatographic purification step with MCS provides increased assurance of safety and product sterility, avoiding

excessive product handling and safety concerns. As endotoxins do not bind to it, the MCS purification step allows a rapid and contaminant free depyrogenation. Gentle binding and elution conditions provide high capacity and product yield. The MCS resin therefore represents a simple, rapid, effective, and cost-saving means for concentration, purification and depyrogenation. In addition, MCS resins can be reused repeatedly.

The inactivated virus may be further purified by gradient centrifugation, preferably density gradient centrifugation. For commercial scale operation a continuous flow sucrose gradient centrifugation would be the preferred option. This method is widely used to purify antiviral vaccines and is known to the expert in the field (*See* JP Gregersen "Herstellung von Virussimpfstoffen aus Zellkulturen" Chapter 4.2 in Pharmazeutische Biotecnologie (eds. O. Kayser and RH Mueller) Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2000.)

The density gradient centrifugation step may be performed using laboratory or commercial scale gradient centrifugation equipment. For example, a swinging bucket rotor, a fixed angle rotor, or a vertical tube rotor, particularly for laboratory scale production of the virus. Preferably, the gradient centrifugation step is performed using a swinging bucket rotor. This type of rotor has a sufficiently long pathlength to provide high quality separations, particularly with multicomponent samples. In addition, swinging bucket rotors have greatly reduced wall effects, and the contents do not reorient during acceleration and deceleration. Because of their longer pathlength, separations take longer compared to fixed angle or vertical tube rotors. The prepared sucrose solutions are controlled via refractometer on their sucrose concentration.

Sucrose gradients for density gradient centrifugation, such as in a swinging bucket centrifuge tubes may be formed prior to centrifugation by the use of a gradient former (continuous/linear). The volume of sample which can be applied to the gradient in a swinging bucket rotor tube is a function of the cross-sectional area of the gradient that is exposed to the sample. If the sample volume is too high, there is not sufficient radial distance in the centrifuge tube for effective separation of components in a multicomponent sample.

An approximate sample volume for swinging bucket rotor SW 28 is 1-5 ml per tube (with a tube diameter of 2.54 cm). The sample is applied to the gradient by pipetting the volume on top of the gradient. The blunt end of the pipette is placed at 45-60° angle to the tube wall, approximately 2-3 mm above the gradient. The sample is injected slowly and allowed to run down the wall of the tube onto the gradient. After centrifugation gradient fractions are recovered by carefully inserting a gauge needle until the bottom of the tube and starting to collect fractions of 2 ml by pumping the liquid from the tube into falcon tubes.

Sucrose density gradients suitable for use with this density gradient centrifugation purification step include 0 – 60%, 5 – 60%, 15 – 60%, 0 – 50%, 5 – 50%, 15 – 50%, 0 – 40%, 5 – 40%, and 15 – 40%. Preferably, the sucrose density gradient is 15 – 40%, 5 – 40% or 0 – 40%.

Alternatively, a discontinuous sucrose density gradient may be used for purification. A discontinuous sucrose density scheme provides for discrete, overlaying layers of differing sucrose concentrations. In one example, a first layer of 50% sucrose is covered by a second layer of 40% sucrose; the second layer is covered by a third layer of 20% sucrose; the third layer is covered by a fourth layer of 10% sucrose; and the fourth layer is covered by the solution containing the virus to be purified.

In one embodiment, inactivated virus is purified by a method comprising a first step of chromatography purification and a second step of gradient centrifugation. Preferably the first step comprises liquid affinity chromatography, such as MCS. Preferably, the second step comprises density gradient centrifugation using a swinging bucket rotor.

Additional purification methods which may be used to purify inactivated SARS virus include the use of a nucleic acid degrading agent, preferably a nucleic acid degrading enzyme, such as a nuclease having DNase and RNase activity, or an endonuclease, such as from *Serratia marcescens*, commercially available as Benzonase™, membrane adsorbers with anionic functional groups (*e.g.* Sartobind™) or additional chromatographic steps with anionic functional groups (*e.g.* DEAE or TMAE). An ultrafiltration/dialfiltration and final sterile filtration step could also be added to the purification method.

Preferably, the purification includes treatment of the SARS viral isolate with one or more nucleic acid degrading enzymes. These enzymes may be used to reduce the level of host cell nucleic acid in the viral purification process. Nucleic acid digesting enzymes for use in cell culture are known in the art and include, for example, Benzonase™.

The treatment of the virus with the nucleic acid degrading enzyme and inactivating agent can be performed by a sequential treatment or in a combined or simultaneous manner. Preferably, the nucleic acid degrading agent is added to the virus preparation prior to the addition of the inactivating agent.

The purified viral preparation of the invention is substantially free of contaminating proteins derived from the cells or cell culture and preferably comprises less than about 1000, 500, 250, 150, 100, or 50 pg cellular nucleic acid / μg virus antigen, preferably less than about 1000, 500, 250, 150, 100, or 50 pg cellular nucleic acid/ dose. Still more preferably, the purified viral preparation comprises less than about 20 pg, and even more preferably, less than about 10 pg. Methods of measuring host cell nucleic acid levels in a viral sample are known in the art. Standardized methods approved or recommended by regulatory authorities such as the WHO or the FDA are preferred.

The invention includes an inactivated vaccine composition comprising a prophylactically effective amount of SARS viral antigen, preferably spike or an immunogenic fragment thereof. The SARS viral antigen is preferably present in a concentration amount of 0.1 to 50 μg

antigen/dose, more preferably 0.3 to 30 μg antigen/dose. Still more preferably, the antigen is about 15 μg/dose.

In one embodiment, a lower concentration of SARS viral antigen is used in inactivated vaccine compositions of the invention. Such lower concentration vaccines may optionally comprise an adjuvant to boost the host immune response to the antigen. In such a "low dose" vaccine, the SARS viral antigen is preferably present in a concentration of less than 15 μg antigen/dose, (*i.e.*, less than 10, 7.5, 5 or 3 μg antigen/dose.

The inactivated vaccine preparations of the invention may further comprise a stabilizer to preserve the integrity of the immunogenic proteins in the inactivated viral preparation. Stabilizers suitable for use in vaccines are known in the art and may include, for example, buffers, sugars, sugar alcohols, and amino acids. Stabilizing buffers are preferably adjusted to a physiological pH range and may include phosphate buffers, Tris buffers, TE (Tris/EDTA), TEN (Tris/NaCl/EDTA) and Earle's salt solution. Stabilizing sugars may include, for example, one or more of saccharose, glucose, fructose, dextranes, dextranesulphate, and trehalose. Stabilizing sugar alcohols may include, for example, Xylite/Xylitole, Mannite/Mannitol, Sorbite/Sorbitol, and Glycerol. Amino acids suitable for use in the invention include, for example, L-glutamine, arginine, cysteine, and lysine. Additional stabilizers which may be used in the invention include Tartaric acid, Pluronic F 68, and Tween 80.

SARS viral isolates which may be used for the inactivated viral preparations of the invention may be obtained and identified by any of the mechanisms described supra. For example, a SARS isolate may be obtained from a clinical sample and plaque purified. Such methods of viral isolation are known in the art.

Further purification procedures can be applied to ensure the seed virus used for preparation of the vaccine does not contain, for example, unwanted adventitious agents. In one embodiment, viral RNA from the viral isolate can be isolated from the virus, purified (and, optionally, the sequence verified through PCR or other means) and then introduced into a suitable cell culture.

As an example of this technique, a clinical viral sample is plaque purified and amplified on vero cells to generate a sufficient amount of the viral sample for analysis. Cellular remnants are then cleared from the supernatant by centrifugation. The virus can then be pelleted by ultracentrifugation and the pellet resuspended in PBS. After further centrifugation purification, the virus containing fraction is treated with a DNase (and optionally also an RNase). Viral RNA is then isolated from this fraction and transfected into a host cell.

Examples 2 and 3 provide an illustration of purification of inactivated whole SARS virus using MCS chromatography resin purification followed by density gradient ultracentrifugation.

Routes and methods of immunization of the vaccines of the invention are discussed in more detail in a section below. Examples 4 and 5 provide illustrations of a mouse immunization scheme with the inactivated SARS virus of the invention.

B. Attenuated SARS Vaccines

The invention includes a composition comprising an attenuated SARS virus. This composition can be used as a prophylactic or therapeutic SARS virus vaccine. Methods of attenuating viruses are known in the art. Such methods include serial passage of the SARS virus in cultured cells (*e.g.*, mammalian cell culture, preferably fetal rhesus kidney cells or VERO cells-see the discussion in Section A above regarding culture of SARS virus), until the SARS virus demonstrates attenuated function. The temperature at which the virus is grown can be any temperature at which with tissue culture passage attenuation occurs. Attenuated function of the SARS virus after one or more passages in cell culture can be measured by one skilled in the art. As used herein, attenuation refers to the decreased virulence of the SARS virus in a human subject. Evidence of attenuated function may be indicated by decreased levels of viral replication or by decreased virulence in an animal model.

Other methods of producing an attenuated SARS virus include passage of the virus in cell culture at sub-optimal or "cold" temperatures and introduction of attenuating mutations into the SARS viral genome by random mutagenesis (*e.g.*, chemical mutagenesis) or site specific directed mutagenesis. Preparation and generation of attenuated RSV vaccines (the methods of which will generally applicable to SARS virus) are disclosed in, for example, EP 0 640 128, US Patent No. 6,284,254, US Patent No. 5,922,326, US Patent No. 5,882,651.

The attenuated derivatives of SARS virus are produced in several ways, such as for example, by introduction of temperature sensitive-mutations either with or without chemical mutagenesis (*e.g.*, 5-fluorouracil), by passage in culture at "cold" temperatures. Such cold adaptation includes passage at temperatures between about 20°C to about 32°C, and preferably between temperatures of about 22°C to about 30°C, and most preferably between temperatures of about 24°C and 28°C. The cold adaptation or attenuation may be performed by passage at increasingly reduced temperatures to introduce additional growth restriction mutations. The number of passages required to obtain safe, immunizing attenuated virus is dependent at least in part on the conditions employed. Periodic testing of the SARS virus culture for virulence and immunizing ability in animals (*e.g.*, mouse, primate) can readily determine the parameters for a particular combination of tissue culture and temperature. The attenuated vaccine will typically be formulated in a dose of from about 10^3 to 10^6 PFU or $TCID_{50}$, or more for maximal efficacy.

Attenuated virus vaccines for SARS-CoV also are produced by creating virus chimeras comprising sequences derived from at least two different coronaviruses, one of which is a SARS-CoV. For example, a virus chimera is produced that comprises nonstructural protein encoding

genes derived from a first coronavirus (e.g., murine, bovine, porcine, canine, feline, avian coronavirus) and one or more structural protein encoding genes (*e.g.*, spike, E, M) from a SARS-CoV. Alternatively, the virus chimera may comprise sequences derived from a human coronavirus that is not a SARS-CoV (*e.g.*, OC43, 229E) together with sequences from a SARS-CoV. Chimeric coronaviruses of the present invention are generated by a variety of methods, including for example allowing for natural RNA recombination in a eukaryotic (*e.g.*, mammalian) cell that contains RNA from each of the parental coronaviruses (*e.g.*, following infection) or by using standard molecular biology techniques known to those of skill in the art to engineer desired virus chimeras (or portions thereof) as cDNA clones, which may then be used to produce infectious virus (see for example, US 6593111 B2; Yount *et al.*, 2003, *Proc. Natl. Acad. Sci. USA* 100(22):12995-13000). An attenuated phenotype of the coronavirus chimeras described herein can be readily measured by one of skill in the art.

Attenuated viruses can be also generated by deleting one or more open reading frames (ORFs) that are not essential for viral replication. Preferably, these deletions occur in the structural region of the genome, such as ORF 3a, 3b, 6, 7a, 7b, 8a, 8b, 9b. See e.g., Haijema BJ, Volders H, Rottier PJ. J Virol. (2004) 78(8):3863-71; and de Haan, C. A., P. S. Masters, X. Shen, S. Weiss, and P. J. Rottier, "The group-specific murine coronavirus genes are not essential, but their deletion, by reverse genetics, is attenuating in the natural host." Virology (2002) 296:177-189. Deletion of such regions within a coronavirus such as SARS can be achieved, for example, by reverse genetics or "targeted recombination" (See, e.g., Masters, P. S., "Reverse genetics of the largest RNA viruses", Adv. Virus Res. (1999) 53:245-264.

Methods of purification of attenuated virus are known in the art and may include one or more of, for instance gradient centrifugation and chromatography. See Gregersen "Herstellung von Virussimpfstoffen aus Zellkulturen" Chapter 4.2 in Pharmazeutische Biotecnologie (eds. O. Kayser and RH Mueller) Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2000.

C. Split SARS Vaccines

The invention includes a composition comprising a split SARS virus formulation and methods for the manufacture thereof. This composition can be used as a prophylactic or therapeutic SARS virus vaccine.

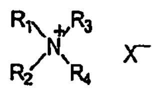
Methods of splitting enveloped viruses are known in the art. Methods of splitting enveloped viruses are disclosed, for example, in WO 02/28422, incorporated herein by reference in its entirety, and specifically including the splitting agents and methods described therein. Methods of splitting influenza viruses are disclosed, for example, in WO 02/067983, WO 02/074336, and WO 01/21151, each of which is incorporated herein by reference in its entirety.

The splitting of the virus is carried out by disrupting or fragmenting whole virus, infectious (wild-type or attenuated) or non-infectious (for example inactivated), with a disrupting

concentration of a splitting agent. The disruption results in a full or partial solubilisation of the virus proteins, altering the integrity of the virus.

Preferably, the splitting agent is a non-ionic or an ionic surfactant. Accordingly, the split SARS virus formulations of the invention may also comprise at least one non-ionic surfactant or detergent. Examples of splitting agents useful in the invention include: bile acids and derivatives thereof, non-ionic surfactants, alkylglycosides or alkylthioglycosides and derivatives thereof, acyl sugars, sulphobetaines, betains, polyoxyethylenealkylethers, N,N-dialkyl-Glucamides, Hecameg, alkylphenoxypolyethoxyethanols, quaternary ammonium compounds, sarcosyl, CTAB (cetyl trimethyl ammonium bromide) or Cetavlon.

Preferably, the ionic surfactant is a cationic detergent. Cationic detergents suitable for use in the invention include detergents comprising a compound of the following formula:



wherein

R_1, R_2 and R_3 are the same or different and each signifies alkyl or aryl, or

R_1 and R_2, together with the nitrogen atom to which these are attached form a 5- or 6-membered heterocyclic ring, and

R_3 signifies alkyl or aryl, or

R_1, R_2 and R_3 together with the nitrogen atom to which these are attached, signify a 5- or 6-membered heterocyclic ring, unsaturated at the nitrogen atom,

R_4 signfies alkyl or aryl, and

X signifies an anion.

Examples of such cationic detergents are cetyltrimethylammonium salts, such as ceytltrimethylammonium bromide (CTAB) and myristyltrimethylammonium salt.

Additional cationic detergents suitable for use in the invention include lipofectine, lipofectamine, and DOT-MA.

Non-ionic surfactants suitable for use in the invention include one or more selected from the group consisting of the octyl- or nonylphenoxy polyoxyethanols (for example the commercially available Triton series), polyoxyethylene sorbitan esters (Tween series) and polyoxyethylene ethers or esters of the general formula :

$$O(CH_2CH_2O)_n\text{-}A\text{-}R$$

wherein n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or phenyl C_{1-50} alkyl; and combinations of two or more of these.

The invention comprises a method of preparing a split SARS virus comprising contacting the SARS virus with a sufficient amount of splitting agent to disrupt the viral envelope. The loss

of integrity after splitting renders the virus non-infectious. Once the disrupted viral envelope proteins are generally no longer associated with whole intact virions, other viral proteins are preferably fully or partially solubilized and are therefore not associated, or only in part associated, with whole intact virions after splitting.

The method of preparing a split SARS virus may further comprise removal of the splitting agents and some or most of the viral lipid material. The process may also include a number of different filtration and/or other separation steps such as ultracentrifugation, ultrafiltration, zonal centrifugation and chromatographic steps in a variety of combinations. The process may also optionally include an inactivation step (as described above) which may be carried out before or after the splitting. The splitting process may be carried out as a batch, continuous, or semi-continuous process.

Split SARS virus vaccines of the invention may include structual proteins, membrane fragments and membrane envelope proteins. Preferably, the split SARS virus preparations of the invention comprise at least half of the viral structural proteins.

One example of a method of preparing a split SARS virus formulation includes the following steps:

(i) propagation of the SARS virus in cell culture, such as MRC-5 cells (ATCC CCL-171), WI-38 cells (ATCC CCL-75), fetal rhesus kidney cells or vero cells (See the discussion in Section A, above, regarding culture of SARS virus);

(ii) harvesting of SARS virus-containing material from the cell culture;

(iii) clarification of the harvested material to remove non-SARS virus material;

(iv) concentration of the harvested SARS virus;

(v) separation of the whole SARS virus from non-virus material;

(vi) splitting of the whole SARS virus using a suitable splitting agent in a density gradient centrifugation step; and

(vii) filtration to remove undesired materials.

The above steps are preferably performed sequentially.

The clarification step is preferably performed by centrifugation at a moderate speed. Alternatively, a filtration step may be used for example with a 0.2μm membrane.

The concentration step may preferably employ an adsorption method, for instance, using $CaHPO_4$. Alternatively, filtration may be used, for example ultrafiltration.

A further separation step may also be used in the method of the invention. This further separation step is preferably a zonal centrifugation separation, and may optionally use a sucrose gradient. The sucrose gradient may further comprise a preservative to prevent microbial growth.

The splitting step may also be performed in a sucrose gradient, wherein the sucrose gradient contains the splitting agent.

The method may further comprise a sterile filtration step, optionally at the end of the process. Preferably, there is an inactivation step prior to the final filtration step.

Methods of preparing split SARS virus formulations may further include treatment of the viral formulation with a DNA digesting enzyme. These enzymes may be used to reduce the level of host cell DNA in the viral purification process. DNA digesting enzymes for use in cell culture are known in the art and include, for example, Benzonase®.

Treatment of the SARS virus formulation with a DNA digesting enzyme may occur at any time in the purification and splitting process. Preferably, however, the SARS virus formulation is treated with a DNA digesting enzyme prior to use of a detergent. Still more preferably, the SARS virus formulation is treated with a DNA digesting enzyme, such as Benzonas, prior to treatment with a cationic detergent, such as CTAB.

Methods of purification of split virus are known in the art. See JP Gregersen "Herstellung von Virussimpfstoffen aus Zellkulturen" Chapter 4.2 in Pharmazeutische Biotecnologie (eds. O. Kayser and RH Mueller) Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2000.

The invention includes a split vaccine composition comprising a prophylactically effective amount of SARS viral antigen, preferably spike or an immunogenic fragment thereof. The SARS viral antigen is preferably present in a concentration amount of 0.1 to 50 μg antigen/dose, more preferably 0.3 to 30 μg antigen/dose. Still more preferably, the antigen is about 15 μg/dose.

In one embodiment, a lower concentration of SARS viral antigen is used in split vaccine compositions of the invention. Such lower concentration vaccines may optionally comprise an adjuvant to boost the host immune response to the antigen. In such a "low dose" vaccine, the SARS viral antigen is preferably present in a concentration of less than 15 μg antigen/dose, (*i.e.*, less than 10, 7.5, 5 or 3 μg antigen/dose.

D. Subunit SARS Vaccines

The invention includes a composition comprising an isolated or purified SARS viral antigen or a derivative thereof. The composition may further comprise one or more adjuvants.

SARS viral antigens can be isolated or purified from a SARS virus grown in cell culture. Alternatively, SARS viral antigens can be recombinantly produced by methods known in the art.

The SARS viral antigens used in the invention can be produced in a variety of different expression systems which are known in the art; for example those used with mammalian cells, baculoviruses, bacteria, and yeast. Such expression systems will typically use polynucleotides encoding the viral antigens of the invention. Such sequences can be obtained using standard techniques of molecular biology, including translating the amino acid sequences listed herein. Accordingly, the invention includes polynucleotides encoding for the viral antigens of the

invention. In addition, the viral antigens of the invention can be produced (at least in part, preferably in whole) via synthetic chemistry methods.

Insect cell expression systems, such as baculovirus systems, are known to those of skill in the art and described in, *e.g.*, Summers and Smith, *Texas Agricultural Experiment Station Bulletin* No. 1555 (1987). Materials and methods for baculovirus/insert cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA. Similarly, bacterial and mammalian cell expression systems are also known in the art and described in, *e.g.*, *Yeast Genetic Engineering* (Barr *et al.*, eds., 1989) Butterworths, London.

A number of appropriate host cells for use with the above systems are also known. For example, mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (*e.g.*, Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Mammalian sources of cells include, but are not limited to, human or non-human primate (*e.g.*, MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75), fetal rhesus lung cells (ATCC CL-160), human embryonic kidney cells (293 cells, typically transformed by sheared adenovirus type 5 DNA), VERO cells from monkey kidneys), horse, cow (*e.g.*, MDBK cells), sheep, dog (*e.g.*, MDCK cells from dog kidneys, ATCC CCL34 MDCK (NBL2) or MDCK 33016, deposit number DSM ACC 2219 as described in WO 97/37001), cat, and rodent (*e.g.*, hamster cells such as BHK21-F, HKCC cells, or Chinese hamster ovary cells (CHO cells)), and may be obtained from a wide variety of developmental stages, including for example, adult, neonatal, fetal, and embryo.

Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include, *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenual polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*.

Nucleic acid molecules comprising nucleotide sequences of the viral antigens or antibodies of the invention can be stably integrated into a host cell genome or maintained on a stable episomal element in a suitable host cell using various gene delivery techniques well known in the art. See., *e.g.*, US Patent No. 5,399,346.

Depending on the expression system and host selected, the molecules are produced by growing host cells transformed by an expression vector under conditions whereby the protein is expressed. The expressed protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth media, the product can be purified directly

from the media. If it is not secreted, it can be isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The invention includes a composition comprising an isolated or purified SARS viral antigen or a derivative thereof. The invention also includes a composition comprising at least two isolated or purified SARS viral antigens or derivatives thereof, which have been co-purified or purified separately and then combined. In one embodiment, the SARS viral antigen is a spike (S) protein. In yet another embodiment, the SARS viral antigen is a nucleocapsid (N) protein, a membrane (M) glycoprotein, or an envelope (E) protein. Preferably, the SARS viral antigen is present in the composition in a purity greater than 75% (*e.g.*, 78%, 80%, 82%, 85%, 88%, 90%, 92%, 95%, 98%).

The invention includes a vaccine composition comprising a prophylactically effective amount of SARS viral antigen, preferably spike or an immunogenic fragment thereof. The SARS viral antigen is preferably present in a concentration amount of 0.1 to 50 μg antigen/dose, more preferably 0.3 to 30 μg antigen/dose. Still more preferably, the antigen is about 15 μg/dose.

In one embodiment, a lower concentration of SARS viral antigen is used in vaccine compositions of the invention. Such lower concentration vaccines may optionally comprise an adjuvant to boost the host immune response to the antigen. In such a "low dose" vaccine, the SARS viral antigen is preferably present in a concentration of less than 15 μg antigen/dose, (*i.e.*, less than 10, 7.5, 5 or 3 μg antigen/dose.

The following example illustrates a method of preparing a SARS virus spike (S) protein subunit vaccine.

SARS virus S antigen may be isolated and purified from a variety of sources and using a variety of methods, including, but not limited to, S antigen expressed in cultured eukaryotic cells (*e.g.*, mammalian cells, such as VERO, CHO) or bacteria (*e.g.*, *E. coli*). Expression of may be achieved by a variety of means, such as, for example, from SARS virus infected cell culture or cell culture supernatants, from cultured cells stably transformed with a DNA expression cassette encoding the SARS virus S protein (*e.g.*, RNA polymerase II promoter operably linked to a SARS virus S gene), or from cultured cells infected with a replication-competent or replication-incompetent virus-based expression vector (*e.g.*, adenovirus vector, poxvirus vector, alphavirus vector, retrovirus vector) encoding the SARS virus S protein, as a means to eliminate the need to work with infectious SARS virus.

<u>1. Subunit SARS Vaccines Produced from SARS Virus Cultures</u>

The SARS virus may be grown in cultured mammalian celle, such as VERO cells, then separated from the cultured cells. A SARS viral antigen, such as the S protein, can then be solubilized and separated from the SARS virus, and further isolated and purified.

In one example, the SARS virus may be produced as described in the Inactivated SARS vaccine examples, then the desired SARS antigen, such as spike protein, may be further purified from the end product using techniques known in the art.

In another example, a SARS subunit vaccine may be produced as follows. SARS virus may be produced using a desired mammalian cell line on microcarrier beads in large, controlled fermentors. For example, vaccine quality African Green Monkey kidney cells (VERO cells) at a concentration of 10^5 cells/mL are added to 60 to 75 L of CMRL 1969 media, pH 7.2, in a 150 L bioreactor containing 360 g of Cytodex-1 microcarrier beads and stirred for 2 hours. Additional CMRL 1969 is added to give a total volume of 150 L. Fetal bovine serum (FBS) is added to a final concentration of 3.5%. Glucose is added to a final concentration of 3.0 g/L and glutamine is added to a final concentration of 0.6 g/L. Dissolved oxygen, pH, agitation and temperature are controlled, and cell growth, glucose, lactate and glutamine levels are monitored. When cells are in logarithmic phases usually on days 3 to 4 reached a density of about 1.0-2.5x10^6 cells/mL, the culture medium is drained from the fermentor and 120 L of CMRL 1969, pH 7.2 (no FBS) is added and the culture stirred for 10 minutes. The draining and filling of the fermentor is usually repeated once but could be repeated up to three times. After washing the cells, the fermentor is drained and 50 L of CMRL 1969 containing 0.1% (v/v) FBS is added. The SARS virus inoculum is added at a multiplicity of infection (m.o.i.) of 0.001 to 0.01. Trypsin may be added to promote efficient infection. Additional CMRL 1969 with 0.1% FBS is added to give a final volume of 150 L. Incubation is continued at 34 C. One viral harvest is obtained from a single fermentor lot, typically at 2-7 days post-infection. Multiple harvests from a single fermentation may also be obtained.

The isolation and purification of S protein may be effected by a variety of means, as described below. For example, collecting S protein-containing flow-through from ion exchange chromatography of solubilized SARS virus envelope proteins; loading the flow through onto a hydroxyapatite matrix, and selectively eluting the S protein from the hydroxyapatite matrix. The selectively eluted S protein may be further concentrated by tangential flow ultrafiltration.

Alternatively, the isolation and purification may be effected by collecting S protein-containing flow-through from ion exchange chromatography of the solubilized SARS virus envelope proteins; loading the flow through onto a hydroxyapatite matrix and collecting an S protein-containing flow through, selectively removing detergent used in the solubilization step from the hydroxyapatite matrix flow through to provide isolated and purified S protein. The isolated and purified S protein may be subsequently concentrated by tangential flow ultrafiltration

Nucleic acid contaminants may be removed from the isolated and purified S protein by treatment with a nucleic acid degrading agent as described above in the Inactivation section. Preferably, the nucleic acid degrading agent is a nuclease, such as for example, Benzonase.

The isolated and purified S protein may be applied to a gel filtration medium and the S protein subsequently collected therefrom to separate the S protein from contaminants of other molecular weights.

Alternatively, the isolation and purification may be effected by loading S protein on a first ion-exchange medium while permitting contaminants to pass through the medium, eluting the S protein from the first ion-exchange medium, to separate the S protein from contaminants of other molecular weights. The eluted S protein is applied to a second ion-exchange medium while allowing contaminants to pass through the second ion-exchange medium. The S protein is subsequently eluted therefrom, to provide the isolated and purified S protein. The eluted S protein may be concentrated by tangential flow ultrafiltration.

Alternatively, substantially pure SARS virus S protein suitable for use as an immunogen in a subunit vaccine formulation may be prepared from infected cell lysates, such as for example using a non-denaturing detergent buffer containing 1% Triton X-100 and deoxycholate to lyse infected cells. The cell lysates are clarified by centrifugation and S protein is purified from the cell lysates by immunoaffinity purification. A monoclonal antibody against the S protein is generated and coupled to beads and a column is constructed with those beads. SARS-infected cell lysates are applied to the column, and the column is washed with PBS containing 0.1% Triton X-100. Protein bound to the column is eluted with 0.1M glycine, pH 2.5, 0.1% Triton X-100. Elution samples are buffered, such as for example, with Tris, and analyzed for the presence of protein. Fractions containing the protein are pooled and dialyzed against PBS

As discussed above, the present invention includes isolated and purified S protein of SARS virus. In one example, the virus is grown on a vaccine quality cell line, such as VERO cells, and the grown virus is harvested. The virus harvest is filtered and then concentrated typically using tangential flow ultrafiltration using a membrane of desired molecular weight cut-off and diafiltered. The virus harvest concentrate may be centrifuged and the supernatant discarded. The pellet from the centrifugation then is detergent extracted to solubilize the S protein, for example, by resuspending the pellet to the original harvest concentrate volume in an extraction buffer containing a detergent such as a non-ionic detergent including TRITON X-100.

Following centrifugation to remove non-soluble proteins, the S protein extract is purified by chromatographic procedures. The extract may first be applied to an ion exchange chromatography column such as a TMAE-fractogel or S-fractogel column equilibrated to permit the S protein to flow through while impurities are retained on the column.

Next, the flow through may be loaded onto a hydroxyapatite column, equilibrated to permit binding of the S protein to the matrix and to permit contaminants to pass from the column. The bound S protein is then eluted from the column by a suitable elutant. The resulting purified solution of S protein may be further processed to increase its purity. The eluate first may be concentrated by tangential flow ultrafiltration using a membrane of desired molecular weight cut-off. The filtrate may be contacted with a polyethylene glycol of desired molecular weight, for example, about 6000 to 8000, to precipitate the protein. Following centrifugation and discard of the supernatant, the pellet may be resuspended in PBS and dialyzed to remove the polyethylene glycol. Finally, the dialyzed solution of S protein may be sterile filtered. The sterile filtered solution may be adsorbed onto alum. The polyethylene glycol precipitation and resuspension purification step may be effected at an earlier stage of the purification operation, if desired.

Alternatively, SARS virus is recovered following growth and harvesting of the virus, and a concentrate obtained such as, for example using PEG precipitation or tangential flow filtration. The virus is contacted with detergent to solubilize the S proteins. Following centrifugation, the supernatant is recovered to further purification of the S protein and the non-soluble proteins discarded.

The supernatant is applied to an ion exchange chromatography column, such as a TMAE-fractogel or S-fractogel column, suitably equilibrated to permit retention of the S protein on the column. The S protein is eluted from the ion-exchange column under suitable conditions. The eluate then may be passed through a gel filtration column, such as a Sephacryl S-300 column, to separate the S protein from contaminants of other molecular weights. A hydroxyapatite column may be employed in place of the Sephacryl column.

The S protein may be eluted from the column to provide a purified solution of S protein. The eluate may be concentrated by tangential flow ultrafiltration using a membrane of desired molecular weight cut-off. The concentrated S protein solution then may be sterile filtered.

Alternatively, viral harvests may be concentrated by ultrafiltration and the concentrated viral harvests may be subjected to an initial purification step, for example, by gel filtration chromatography, polyethylene glycol precipitation or Cellufine sulfate chromatography. The purified virus may then be detergent extracted to solubilize the S protein. Following solubilization of the S protein, the supernatant may be loaded onto an ion-exchange column such as Cellufine sulfate chromatography column equilibrated to permit the protein to bind to the column while permitting contaminants to flow through. Similarly, a TMAE-fractogel or S-fractogel column may be used in place of the Cellufine sulfate column. The two columns also may be combined in sequential purification steps. The S protein is eluted from the columns to

provide a purified solution of the protein. This solution may be concentrated by tangential flow ultrafiltration using a membrane of desired molecular weight cut-off and diafiltered.

Specifically, in one method of S protein purification, the virus harvest concentrate is centrifuged at 28,000 x g for 30 minutes at 4 C. The supernatant is discarded and the pellet resuspended in extraction buffer consisting of 10 mM Tris-HCl, pH 7.0, 150 mM NaCl, 2% (w/v) Triton X-100 to the original harvest concentrate volume. Pefabloc is added to a final concentration of 5 mM. The suspension is stirred at room temperature for 30 minutes. The supernatant, containing the soluble S protein, is clarified by centrifugation at 28,000 x g for 30 minutes at 4 C. A TMAE--Fractogel column is equilibrated with 10 mM Tris-HCl, pH 7.0, 150 mM NaCl containing 0.02% Triton X-100. The Triton X-100 supernatant, containing the soluble S protein, is loaded directly onto the TRAE-Fractogel column. The total volume added plus 2 bed volumes of 10 mM Tris-HCl, pH 7.0, 150 mM NaCl containing 0.02% Triton X-100 are collected. The TMAE--Fractogel flow-through containing S protein is diluted 3-fold with 10 mM Tris-HCl, pH 7.0, containing 0.02% Triton X-100.

An hydroxyapatite column is equilibrated with 10 mM Tris-HCl, pH 7.0, 50 mM NaCl, 0.02% Triton X-100. After loading the TMAE flow-through, the column is washed with 2 column volumes of 10 mM Tris-HCl, pH 7.0, 50 mM NaCl , 0.02% Triton X-100 followed by 4 column volumes of 5 mM sodium phosphate, pH 7.0, 1M NaCl, 0.02% Triton X-100. The proteins are eluted with 4 column volumes of 20 mM sodium phosphate, pH 7.0, 1M NaCl , 0.02% Triton X-100. Fractions are collected based on A280 and the protein content and antigen concentrations are measured. The purified S protein is ultrafiltered by tangential flow ultrafiltration using a 300 kDa NMWL membrane.

2. Recombinant Production of Subunit SARS Vaccines

As discussed above, SARS virus proteins may be produced by recombinant expression. Host cells suitable for recombinant expression include bacterial, mammalian, insect, yeast, *etc.* Recombinant expression may be used to produce a full length SARS protein, a fragment thereof, or a fusion therewith.

Fusion peptides may be used to facilitate the expression and purification of the recombinant SARS protein. For example, recombinant production of the SARS polypeptides can be facilitated by the addition a tag protein to the SARS antigen to be expressed as a fusion protein comprising the tag protein and the SARS antigen. Such tag proteins can facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-tag,, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiantion factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide

isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of tag proteins can be found at Terpe *et al.*, "Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems", Appl Microbiol Biotechnol (2003) 60:523-533.

After purification, the tag proteins may optionally be removed from the expressed fusion protein, *i.e.*, by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor X_a.

Accordingly, the invention further includes a SARS virus subunit vaccine comprising a fusion protein. Preferably, the fusion protein comprises a first amino acid sequence encoded by a SARS virus polynucleotide sequence. SARS virus polynucleotide sequences which may encode said first amino acid sequence include one or more of the SARS virus polynucleotide sequences identified in this application and fragments thereof.

The fusion protein may comprise an amino acid sequence of a SARS virus protein or a fragment thereof. Said SARS virus protein may be selected from one or more of the group consisting of the following SARS virus proteins: P28, P65, Nsp1, Nsp2 (3CL protease), Nsp3, Nsp3, Nsp4, Nsp 5, Nsp6, Nsp 7, Nsp 8, Nsp 9 (RNA polymerase), Nsp 10 (helicase), Nsp 11, Nsp 12, Nsp 13, Spike, Orf 3, Orf 4, Envelope, Matrix, Orf 7, Orf 8, Orf 9, Orf 10, Orf 11, Nucleocapsid and Orf 13.

In one embodiment, the fusion protein comprises a first amino acid sequence comprising a SARS virus antigen or a fragment thereof. Said SARS virus amino acid sequence may comprise one or more of the T-epitope sequences identified above.

Preferably, the fusion protein comprises an amino acid sequence of a SARS virus spike protein, or a fragment thereof. Specific fragments of the spike protein which may be used in the fusion protein include the S1 domain and the S2 domain. Further fragments of the spike protein which may be used in the fusion protein include regions of each of the S1 and S2 domains, including the receptor binding region of the S1 domain, the oligomerization domain regions of the S2 domain, the leucine zipper regions of the S2 domain, the membrane anchor region of the S2 domain, the hydrophobic domain region of the S2 domain, the cystein-rich domain region of the S2 domain, and the cytoplasmic tail region of the S2 domain. (See FIGURE 19). Amino acid sequences of the Spike protein corresponding to these regions can be identified by those skilled in the art, including, for example, using the functional predictions set forth earlier in the application (predicted transmembrane helices, predicted N-terminus signaling regions, predicted coiled-coil regions, *etc.*) as well as by homology comparison to the sequences of other known Coronaviruses (See FIGURES 4F and 5).

The fusion protein may further comprise a second amino acid sequence. Said second amino acid sequence may comprise a polypeptide sequence which facilitates protein expression

or purification, preferably one of the tag sequences discussed above. Alternatively, said second amino acid sequence may comprise a second amino acid sequence from a SARS virus. Alternatively, said second amino acid sequence may comprises an amino acid sequence from another virus or bacteria, including one or more of the viruses or bacteria identified in Section I, below.

Said second amino acid sequence may comprise an amino acid sequence from another respiratory virus. Said second amino acid sequence may comprise an amino acid sequence from a virus selected from the group consisting of coronavirus, influenza virus, rhinovirus, parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, and metapneumovirus.

In one embodiment, said second amino acid sequence may comprise an amino acid sequence from an adjuvant, including one or more of the adjuvants identified in section I, below.

In one embodiment, the invention includes a fusion protein comprising an amino acid sequence of a SARS virus spike protein or a fragment thereof. The fusion protein may further comprise a second amino acid sequence comprising an amino acid sequence selected from the group consisting of a second SARS virus protein, a non-SARS virus protein, a bacterial protein, and an adjuvant.

(a) Bacterial Expression of Subunit SARS Vaccines

In one embodiment, bacterial host cells are used for recombinant expression of SARS virus proteins. Bacterial host cells suitable for use in the invention include, for example, *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*

The SARS viral protein may be modified to facilitate bacterial recombinant expression. In particular, the SARS spike protein may be modified to facilitate transport of the spike protein to the surface of the bacterial host cell.

Applicants have discovered that there is strong structural homology between the SARS virus spike protein and the NadA protein of *Neisseria meningitidis*. Both proteins have an N-terminal globular "head" domain (amino acids 24-87), an intermediate alpha-helix region with high propensity to form coiled-coil structures (amino acids 88-350), and a C-terminal membrane anchor domain formed by four amphipatix transmembrane beta strands (amino acids 351-405 of NadA). In addition, a leucine zipper motive is present within the coiled-coil segment. See, FIGURE 19 depicting the SARS spike protein structure Comanducci *et al.*, "NadA, a Novel Vaccine Candidate of Neisseria meningitidis", J. Exp. Med. 195 (11): 1445-1454 (2002). In addition, a leucine zipper motif of NadA is present within the coiled-coil segment. The NadA protein also forms high molecular weight surface-exposed oligomers (corresponding to three or four monomers) anchored to meningococcal outer membrane.

When the NadA protein is expressed in *E. coli*, the full-length protein is assembled in oligomers anchored to the outer membrane of *E. coli*, similar to the way the protein is presented

in meningococcus. The NadA protein devoid of the predicted membrane anchor domain is then secreted into the culture supernatant. This secreted protein is soluble and still organized in trimers.

The invention therefore includes a fusion protein comprising an amino acid sequence of a SARS virus spike protein or a fragment thereof and a second amino acid sequence of a bacterial adhesion protein or a fragment thereof. Preferably, said adhesion protein is selected from the group consisting of NadA, YadA (of enteropathogenic *Yersinia*), and UspA2 (of *Moraxella catarrhalis*). Additional NadA-like proteins include serum resistance protein DsrA of *Haemophilus ducreyi*, the immunoglobulin binding proteins EibA, C, D, and F of *E. coli*, outer membrane protein 100 of *Actinobacillus actinomycetemcomitans*, the *saa* gene carried on the large virulence plasmid present in shiga toxigenic strains of *E. coli* (STEC), and each of the bacterial adhesion proteins described in U.K. Patent Application No. 0315022.4, filed on June 26, 2003, each of which are specifically incorporated herein by reference.

Preferably, said adhesion protein comprises NadA or a fragment thereof.

Such fusion proteins may be used to facilitate recombinant expression of immunogenic portions of SARS surface antigens, such as spike. These fusion constructs may also allow the SARS S1 and/or S2 domains to adapt to a native confirmation. These fusion proteins are also able to oligomerize and form dimers or trimers, allowing the S1 and/or S2 domains to associate and adapt conformations as in the native SARS spike protein. Further, these expression constructs facilitate surface exposure of the SARS spike protein.

The fusion proteins of the invention preferably comprise a leader peptide from a NadA like protein, preferably NadA, a polypeptide from the immunogenic "head" region of the spike protein, and a stalk region from either the NadA like protein or the Spike protein. During expression and processing of the fusion protein, one or more amino acids may be cleaved off or removed, such as, *i.e.*, the leader peptide or a membrane anchor domain.

The stalk regions facilitate oligomerization of the expression protein. Optionally, the fusion proteins of the invention further include an anchor region of a NadA like protein. This anchor region allows the expression fusion protein to anchor and assemble on the bacterial cell surface.

The fusion proteins of the invention include the following constructs:

(i) the NadA leader peptide (optionally also including the first 6 amino acids of the mature NadA protein to facilitate processing of the leader peptide and appropriate maturation of the protein) followed by the Spike S1 domain. Preferably, this construct comprises amino acids 1-29 of NadA (corresponding to the NadA leader peptide and the first 6 amino acids of the mature NadA protein, as shown in FIGURE 22 and as set forth below) followed by amino acids 14-662

of a SARS virus Spike protein (corresponding to the S1 domain, see FIGURE 19 and SEQ ID NO: 6042 and as set forth below). Specifically, construct (i) comprises SEQ ID NO: 7302.

(ii) the NadA leader peptide (optionally also including the first 6 amino acids of the mature NadA protein to facilitate processing of the leader peptide and appropriate maturation of the protein) followed by the Spike S1 domain, followed by the stalk and anchor membrane domains of NadA. Preferably, this construct comprises amino acids 1-29 of NadA (corresponding to the NadA leader peptide and the first 6 amino acids of the mature NadA protein, as shown in FIGURE 22 and as set forth below) followed by amino acids 14-662 of a SARS virus Spike protein (corresponding to the S1 domain, see FIGURE 19 and SEQ ID NO: 6042 and as set forth below) followed by amino acids 88-405 of NadA (corresponding to the stalk and the anchor membrane domains). Specifically, construct (ii) comprises SEQ ID NO: 7303.

(iii) the NadA leader peptide (optionally also including the first 6 amino acids of the mature NadA protein) followed by a SARS virus Spike S1 domain, followed by the NadA stalk domain. Preferably, this construct comprises amino acids 1-29 of NadA followed by amino acids 14-662 of a SARS virus Spike protein (corresponding to the S1 domain), followed by amino acids 88-350 of NadA (corresponding to the stalk domain). Specifically, construct (iii) comprises SEQ ID NO: 7304.

(iv) the NadA leader peptide (optionally also including the first 6 amino acids of the mature NadA protein), followed by a SARS virus Spike S1 and S2 domain (excluding the putative transmembrane region), followed by the anchor domain of NadA. Preferably, this construct comprises amino acids 1-29 of NadA, followed by amino acids 14-1195 of a SARS virus Spike protein (corresponding to S1 and S2, excluding the putative transmembrane region), followed by amino acids 351-405 of NadA (corresponding to the NadA anchor domain). Specifically, construct (iv) comprises SEQ ID NO: 7305. Alternatively, the NadA anchor domain may comprise amino acids 332 – 405 of NadA.

(v) the NadA leader peptide (optionally also including the first 6 amino acids of the mature NadA protein), followed by a SARS virus Spike S1 and S2 domain (exclusing the putative transmembrane region). Preferably, this construct comprises amino acids 1-29 of NadA, followed by amino acids 14-1195 of a SARS virus Spike protein. Specifically, construct (v) comprises SEQ ID NO: 7306.

In each of constructs (i) to (v), the first 23 amino acids are the NadA leader peptide, and the GS dipeptide at residues 679-680 arises from the insertion of a restriction enzyme site.

In constructs (i), (ii) and (iii), the NadA "head" is replaced by the Spike S1 domain, and the fusion proteins are anchored to the outer membrane of *E.coli* or secreted in the culture supernatant, respectively. In constructs (iv) and (v), the "head" and "stalk" domains of NadA are

replaced by S1 and S2 Spike domains; also in this case, the two fusion proteins are anchored to the outer membrane of *E.coli* or secreted in the culture supernatant, respectively.

Accordingly, the invention further includes a fusion protein comprising an amino acid sequence of a SARS virus spike protein or a fragment thereof and a second amino acid sequence of a bacterial adhesion protein or a fragment thereof. Preferably, amino acids corresponding to the "head" of the adhesion protein are replaced by amino acids corresponding to a SARS virus Spike S1 domain. Alternatively, the amino acids corresponding to the "head" and "stalk" domains of the bacterial adhesion protein are replaced by amino acids corresponding to the SARS virus spike protein S1 and S2 domains.

As discussed above and shown in Figure 19, the S1 domain of the Spike protein is identified as the globular receptor binding "head" region. The S1 domain of the Spike protein preferably comprises about amino acids 14-662 of SEQ ID NO: 6042. The S1 domain may comprise a shorter amino acid sequence, wherein amino acids are removed from either the N-terminal or C-terminal regions. Preferably, 3, 5, 7, 9, 13, 15, 20 or 25 amino acids are removed from either the N-terminal or C-terminal regions. The S1 domain further includes amino acid sequences having sequence identity to the S1 region of SEQ ID NO: 6042. An example of the S1 domain is SEQ ID NO: 7307:

As discussed above and shown in Figure 19, the S2 domain of the Spike protein is identified as the "stalk" region. The "stalk" region comprises oligomerization domain regions, a leucine zipper domain regions, membrane anchor regions, hydrophobic domain regions, cystein-rich domain region and a cytoplasmic tail region. The S2 domain of the Spike protein preferably excludes the transmembrane region and comprises about amino acids 663-1195 of SEQ ID NO: 6042. The S2 domain may comprise a shorter amino acid sequence, wherein amino acids are removed from either the N-terminal or C-terminal regions. Preferably, 3, 5, 7, 9, 13, 15, 20 or 25 amino acids are removed from either the N-terminal or C-terminal regions. The S2 domain further includes amino acid sequences having sequence identity to the S2 region of SEQ ID NO: 6042. An example of the S1 domain (with the transmembrane region excluded) is SEQ ID NO: 7308.

An example of the NadA protein described above is SEQ ID NO: 7309. As discussed above, the leader sequence of NadA used in the fusion protein preferably comprises about the first 29 amino acids of NadA (including a leader sequence with about 6 amino acids of the NadA head protein). Examples of such a leader sequences are set forth as SEQ ID NOS: 7310 and 7311 below. The fusion protein may use a leader sequence comprising a shorter amino acid sequence, wherein amino acids are removed from either the N-terminal or C-terminal regions. Preferably, 1, 2, 3, 4, or 5 amino acids are removed from either the N-terminal or C-terminal end of the sequence. The leader sequence used in the fusion protein may also include an amino acid

sequences having sequence identity to SEQ ID NO: 7310 or SEQ ID NO: 7311. Preferably, the leader sequence comprises SEQ ID NO: 7311.

Optionally, the fusion peptide comprises about the first 6 amino acids of the mature NadA protein to facilitate processing of the leader peptide and appropriate maturation of the protein. An examples of the first 6 amino acids of a mature NadA proteins is SEQ ID NO: 7312..

As discussed above, the stalk and anchor sequences of NadA used in the fusion protein preferably comprise about amino acids 88-405 of NadA. An example of an amino acid sequence comprising NadA stalk and anchor regions is set forth below as SEQ ID NO: 7313 below. An example of an amino acid sequence comprising a NadA stalk region (without the anchor region) is set forth as SEQ ID NO: 7314 below. An example of an amino acid sequence comprising a NadA anchor region is set forth as SEQ ID NO: 7315 below. The fusion protein may use a stalk (and/or anchor) sequence comprising a shorter amino acid sequence, wherein amino acids are removed from either the N-terminal or C-terminal regions. Preferably, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acids are removed from either the N-terminal or C-terminal end of the sequence. The leader sequence used in the fusion protein may also include an amino acid sequences having sequence identity to the SEQ ID NO: 7313.

The fusion proteins of the invention, including those described above, may be prepared, for example, as follows. Single fragments (such as the regions described above) may be amplified by PCR using the oligonucleotide primers set forth in the Table below. ($S1_L$ refers to the Spike protein fused to the leader peptide of NadA; S2 refers to the stalk region of the Spike protein, with and without the stop codon). The oligonucleotides were designed on the basis of the DNA sequence of NadA from *N. meningitidis* B 2996 strain and of Spike from SARS virus isolate FRA1. Each oligonucleotide includes a restriction site as a tail in order to direct the cloning into the expression vector pET21b.

		SEQ ID NO:	Restriction site
$S1_L$	For	7316	NdeI
$S1_L$	Rev	7317	BamHI
S2	For	7318	BamHI
S2	Rev	7319	HindIII
S2-stop	Rev	7320	XhoI
$NadA_{88}$	For	7321	BamHI
$NadA_{350}$	Rev	7322	XhoI
$NadA_{332}$	For	7323	HindIII
$NadA_{405}$	Rev	7324	XhoI

The single fragments are sequentially cloned into pET21b vector, in order to express the proteins under the control of inducible T7 promoter. The S1 domain of the Spike protein fused to the leader peptide of NadA ($S1_L$) was obtained by PCR using the primers $S1_L$-For and $S1_L$-Rev. The forward oligonucleotide primer contains the NdeI restriction sequence and the

sequence coding for the leader peptide of NadA plus the first 6 aminoacids of the mature protein. The PCR fragment was cloned as a NdeI/BamHI fragment in the pET21b vector opened with the same restriction enzymes. This clone (pET-S1_L) was then used to sequentially clone the other different domains, as BamHI/XhoI, BamHI/HindIII or HindIII/XhoI fragments. BamHI and HindIII restriction sites introduce the aminoacids GS and KL, respectively.

The PCR amplification protocol was as follows: 200ng of genomic DNA from *Neisseria meningitidis* 2996 or 10 ng of plasmid DNA preparation (plasmid pCMVnew, containing the entire gene coding of the Spike protein), were used as template in the presence of 40μM of each oligonucletide primer, 400-800 μM dNTPs solution, 1x PCR buffer (including 1.5mM $MgCl_2$), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaQ or Invitrogen Platinum Pfx DNA polymerase).

After a preliminary 3 minute incubation of the whole mix at 95°C, each sample underwent a two-step amplification: the first 5 cycles were performed using the hybridisation temperature that excluded the restriction enzyme tail of the primer (Tm1). This was followed by 30 cycles according to the hybridisation temperature calculated for the whole length oligos (Tm2). Elongation times, performed at 68°C or 72°C, varied according to the length of the fragment to be amplified.The cycles were completed with a 10 minute extension step at 68°C or 72°C.

The amplified DNA was either loaded directly on agarose gel and the DNA fragment corresponding to the band of correct size was purified from the gel using the Qiagen™ Gel Extraction Kit, following the manufacturer's protocol.

The purified DNA corresponding to the amplified fragment and the plasmid vectors were digested with the appropriate restriction enzymes, purified using the QIAquick™ PCR purification kit (following the manufacturer's instructions) and ligation reactions were performed.

The ligation products were transformed into competent *E. coli* DH5α and screening for recombinant clones was performed by growing randomly-selected colonies and extracting the plasmid DNA using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions.

Recombinant plasmids were introduced into *E. coli* BL21(DE3) used as expression host. Single recombinant colonies were inoculated into LB + ampicillin and incubated at 37°C for 14-16 h. Bacteria were directly recovered by centrifugation (uninduced conditions) or diluted in fresh medium and grown at 37°C until OD_{600} between 0.4-0.8. Protein expression was induced by addition of 1 mM Isopropyl-1-thio-β-D-galactopyranoside (IPTG) for three hours (induced conditions).

Whole cell lysates were obtained resuspending bacteria in SDS-sample buffer 1X and boiling for 5-10 min. Equal amounts of proteins were separated using NuPAGE (Invitrogen) or

BIORAD Gel System, according to the manufacturer's instructions. Proteins were revealed by Coomassie-blue staining or transferred onto nitrocellulose membranes for western blot analysis. Western blot was performed using a rabbit polyclonal anti-serum against purified $NadA_{\Delta 351\text{-}405}$ (diluted 1:3000) and a secondary peroxidase-conjugate antibody (DAKO).

Results of the expression in *E.coli* of $S1_L$, $S1_L$-NadA and $S1_L$-$NadA_{\Delta anchor}$ are shown in FIGURES 38 and 39. Schematics of the fusion constructs are shown in FIGURE 37.

Bacterial expression of the SARS viral antigens may also be used to prepare compositions comprising outer membrane vesicles wherein said outer membrane vesicles comprise one or more SARS viral antigens.

Outer Membrane Vesicles ("OMV"), also referred to as blebs, refer to vesicles formed or derived from fragments of the outer membrane of a Gram negative bacterium. OMVs typically comprise outer membrane proteins (OMPs), lipids, phospholipids, periplasmic material and lipopolysaccharide (LPS). Gram negative bacteria often shed OMVs during virulent infections in a process known as blebbing. OMVs can also be obtained from Gram negative bacteria via a number of chemical denaturation processes, such as detergent extraction. Synthetic OMVs or liposomes, comprising a lipid bilayer and typically enclosing an aqueous core, can also be prepared with the SARS viral antigens of the invention.

The OMVs of the invention are preferably lipid vesicles comprising a lipid bilayer surrounding an aquous core. Typically the lipid vesicles are of unilamellar structure (*i.e.*, a single lipid bilayer surrounds the aquous core), although multilammelar lipid vesicles may also be used in the compositions of the invention. OMVs typically have sizes in the nanomolar to micromolar range, *e.g.*, from 1 nM to 100 μM, more typically from 10nM to 10 μM and preferably from 30 nM to 1 μM.

The OMVs of the invention are preferably prepared from gram negative bacteria. Gram negative bacteria are those bacteria that fail to resist decolorization in the commonly known Gram staining method. Gram negative bacteria are characterized by a complex multilater cell wall and often possess an outer layer polysaccharide capsule. Gram negative bacteria suitable for producing OMVs include, for example, species from *Neisseria*, *Moraxella*, *Kingella*, *Acinetobacter*, *Brucella*, *Bordetella*, *Chlamydia*, *Porphyromonas*, *Actinobacillus*, *Borelia*, *Serratia*, *Campylobacter*, *Helicobacter*, *Haemophilus*, *Escherichia*, *Legionella*, *Salmonella*, *Pseudomonas* and *Yersinia*.

The OMVs of the invention preferably comprise one or more SARS viral antigens or a fragment thereof. The SARS viral antigens may be recombinantly expressed in a Gram negative bacterial host cell and then harvested with the OMV.

Antigenic components, such as recombinantly expressed SARS viral antigens, may be located in any or all of the three main compartments of the lipid vesicles, including attached to

either the interior or exterior surface of the lipid vesicle, for example via a membrane anchor domain, or attachment to a lipid moiety; inserted into the lipid bilayer, for example where the antigenic component is itself a hydrophobic or lipid based entity; or located within the aqueous center or core of the lipid vesicle.

Synthetically prepared OMVs, or liposomes, may be used in the invention. Such liposomes may comprise a number of different lipids and fatty acids. Suitable lipids for inclusion in liposomes of the invention include but are not limited to phophatidylinositol-(4,5)-diphosphate, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, phosphatidyglycerol, cholesterol, beta-oleolyl-gamma-palmitoyl, lipopolysaccharides and galactocerbrosides.

Suitable means for extraction of OMVs from bacterial sources include deoxycholate extraction, Tris/HCl/EDTA extraction, and lithium acetate extraction. Preferably, the extraction process comprises a physical and/or chemical means to disrupt the bacterial cell outer membrane in order to release sufficient OMVs for purification and isolation. *See, e.g.*, WO 03/051379.

The OMVs of the invention may be enriched and/or supplemented with antigenic components, such as SARS viral antigens, by methods known in the art, including, for example, direct combination *in vitro* where an energetic combination step can optionally be applied to facilitate integration of the antigenic component into a compartment of the liposome. Methods of energetic combination suitable for use in the invention include homogenization, ultrasonication, extrusion, and combinations thereof.

Preferably, the antigenic component, such as the SARS viral antigen, is recombinantly produced by the host cell from which the OMV is derived. In one embodiment, such OMVs are prepared by introducing nucleic acid sequence encoding for the SARS viral antigen into the recombinant host cell. Preferably the nucleic acid sequence encoding for the SARS viral antigen is controlled by a strong promoter sequence. Preferably, the nucleic acid sequence encoding the SARS viral antigen further comprises an outer-membrane targeting signal. For example, the nucleic acid sequence encoding the SARS viral antigen may be fused to a sequence encoding for a naturally occurring outer membrane protein of the bacterial host. Preferably, the nucleic acid sequence encoding the SARS viral antigen is fused to the signal peptide sequence of the naturally occurring outer membrane protein of the bacterial host.

Methods of preparing an optimizing OMVs for use in vaccines are disclosed in, for example Filip *et al.*, *J. Bact.* (1973) 115: 717-722; Davies *et al.*, *J. Immunol. Method* (1990) 143:215-225; and WO 01/09350.

In one embodiment, a bacterial host cell, such as *E. coli*, are transformed to express the SARS spike protein. As discussed above, the spike protein may be modified to facilitate bacterial expression and transport of the spike protein to the surface of the host cell. Each of the

Spike/NadA fusion constructs discussed above may be used in the OMV preparations of the invention. Preferably, constructs comprising the spike S1 globular head domain fused to the stalk region of NadA are used to generate OMVs. The construct may optionally include the NadA leader peptide as well as the NadA anchor peptide. Schematic diagrams of these preferred OMV constructs are depicted in FIGURE 49.

Example 6 describes one method of preparing the OMVs of the invention.

(b) Mammalian Expression of Subunit SARS Vaccine

As discussed above, mammalian host cells may be used for recombinant expression of SARS virus proteins. Mammalian host cells suitable for use in the invention include, for example, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (*e.g.*, Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Mammalian sources of cells include, but are not limited to, human or non-human primate (*e.g.*, MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75), human embryonic kidney cells (293 cells, typically transformed by sheared adenovirus type 5 DNA), VERO cells from monkey kidneys (including, for example COS7 cells), horse, cow (*e.g.*, MDBK cells), sheep, dog (*e.g.*, MDCK cells from dog kidneys, ATCC CCL34 MDCK (NBL2) or MDCK 33016, deposit number DSM ACC 2219 as described in WO 97/37001), cat, and rodent (*e.g.*, hamster cells such as BHK21-F, HKCC cells, or Chinese hamster ovary cells (CHO cells)), and may be obtained from a wide variety of developmental stages, including for example, adult, neonatal, fetal, and embryo.

The polynucleotides encoding the SARS viral proteins may be modified to facilitate or enhance expression. For example, commercial leader sequences known in the art, such as tPA or IgK or interleukin-2, may be used in the recombinant constructs. Preferably, however, the natural SARS leader sequence is used. Use of the natural leader sequence can be used to ensure that the protein will be trafficked in human cells in the same way as during a normal viral infection, which may be advantageous *e.g.* for DNA vaccines, where antigen is expressed *in situ*.

As discussed above, tag sequences can be used in the expression constructs to facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-tag,, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiantion factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of tag proteins can be found at Terpe *et al.*, "Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems", *Appl Microbiol Biotechnol* (2003) 60:523-533.

After purification, the tag proteins may optionally be removed from the expressed fusion protein, *i.e.*, by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor X_a.

One or more amino acid sequences or amino acid domains of the spike protein may be removed to facilitate mammalian recombinant expression. For instance, the entire S2 domain or the spike transmembrane region may be removed. Representative examples of some expression constructs of both full length and truncated spike glycoprotein suitable for mammalian expression are shown in FIGURE 40. Polynucleotide sequences representing each construct are shown in SEQ ID NOS 6578-6583. A description of each annotation is shown below:

Clone Name	Description	Expression Construct
nSh	natural leader sequence full length Spike histidine tag	SEQ ID NO: 6578
nS	natural leader sequence full length Spike	SEQ ID NO: 6579
nShΔTC	natural leader sequence Spike without transmembrane sequence histidine tag	SEQ ID NO: 6580
nSΔTC	natural leader sequence Spike without transmembrane sequence	SEQ ID NO: 6581
nS1h	natural leader sequence S1 domain histidine tag	SEQ ID NO: 6582
nS1	natural leader sequence S1 domain	SEQ ID NO: 6583

Cloned cDNA fragments that encompass full-length Spike coding sequences, as well as a Spike construct deleted of the transmembrane and cytoplasmic domains (TM-Cy-deleted Spike) for secretion were inserted into an expression vector pCMVIII to create nSh and nShΔTC, respectively. Both spike proteins were tagged with six histidine residues at the end of C-terminus to aid initial characterization of the expressed spike proteins. Similar sequences encoding full-length Spike or transmembrane and cytoplasmic domain deleted Spike, but without the histidine "tag" are readily substituted by one of skill in the art.

The likely locations of the expressed spike constructs was assessed by separating expressed proteins into an aqueous fraction (AF) and a detergent fraction (DF) using the procedure shown in Figure 48, with results of western blot analysis shown in Figure 43. The above described vector constructs were evaluated for expression after transfection into COS7 cells. The construct expressing the full length spike protein remained in the cell membrane while the construct expressing the truncated spike protein was located either in the cytosol (Figure 43) or secreted into the cell medium (Figure 44). As shown in Figure 43, full-length spike protein is found in DF (membrane) in an aggregated form, while the truncated protein is found in AF (cytosol) as a

monomer. As shown in Figure 44, deleted proteins (ShΔTC) are secreted, and a small fraction of full-length spike protein is detected in the medium by rabbit serum.

Recombinantly expressed spike proteins may be oligomerized. When the spike proteins are to be used in a vaccine or to generate antibodies specific to the spike protein, they are preferably oligomerized. In order to obtain oligomerized spike protein, it is preferred to maintain the transmembrane domain in the recombinant expression construct. For example, FIGURE 41 illustrates a western blot of COS7 cell lysates comparing expressed nSh and nShΔTC using both anti-his tag and rabbit anti-SARS antibodies. As shown full-length (nSh) aggregates, but the truncated (nShΔTC) spike protein does not. Antibody raised against the His-tagged protein recognizes full-length and truncated spike proteins in native and reduced forms. Rabbit antiserum recognizes spike protein only in non-reducing conditions. Spike aggregates or oligomers were present in larger amounts in the cell lysates from the expressed nSh constructs. Preferably, the oligomerized spike proteins form a homotrimer, as indicated in FIGURE 47

A further experiment, illustrated in FIGURE 42, demonstrates that the oligomerization of the expressed nSh constructs is likely due to a non-covalent linkage (and is likely not due to, for example, a disulfide bond). The oligomer dissociates into monomers at elevated temperature (80-100°C), but is stable in reducing conditions if not heated.

It is further preferred that recombinantly expressed spike proteins are glycoslyated. Tunicamycin and glycosidases were used to assess glycosylation. FIGURE 45 illustrates that glycoslation of expressed spike proteins is not affected by removal of the transmembrane domain region. Both full-length (Sh) and truncated (ShΔTC) SARS spike proteins are glycosylated.

Preferably, expression of the constructs of the invention is not toxic to the mammalian host cell. FIGURE 46 demonstrates that expression of the illustrated spike constructs is not toxic to the COS7 host cell.

Methods for transfecting, expressing, culturing, isolating and purifying recombinant proteins from mammalian cell cultures are known in the art. For example, the SARS spike constructs of the invention may be expressed in 293 cells. These cells may be cultured and transfected in static or monolayer cultures. For rapid large-scale production of SARS protein antigens in sufficient quantities for *in vitro* and *in vivo* evaluation, including immunogenicity studies, large-scale transient transfection of 293 (human embryonic kidney) cells may be used to obtain milligram quantities of the recombinant antigen(s). Alternatively, larger scale transfection of these cells may be performed with 293 cells in suspension culture. Preferably, the expressed SARS proteins are harvested from the transfected cells between 48 and 72 hours after transfection or even from 72 to 96 or more hours after transfection.

Where the host cells are transfected with truncated spike expression constructs, the expressed spike protein is secreted from the host cells and collected from the cell media. After

concentration, the spike protein may be purified from the media using, for example, GNA lectin followed by DEAE and ceramic hydroxyapatite column chromatography.

Where the host cells are transfected with full length spike expression constructs, but rather is retained within the cells, and may be purified from triton X-100 detergent extracted cells. The full-length Spike protein can then be captured on GNA lectin, followed by hydroxyapatite and SP chromatography.

Chinese Hamster Ovary (CHO) or other eukaryotic (*e.g.*, mammalian) cells that stably express the SARS viral antigens of the invention may also be derived (*e.g.* Figure 73). Preferably, the cells are CHO cells, and these constructs will comprise one or more marker or selection genes in order to select for the desired CHO cells. In one embodiment, the constructs comprise a CMV enhancer/promoter, ampicillin resistance gene, and a fused DHFR and attenuated neomycin gene for selection purposes. Stable cell lines can then be produced using the neomycin selection system in CHOK-1 cells. Selected clones can then be sequenced to verify the integrity of the insert, and transient transfections can then be performed using Trans-LT1 polyamine transfection reagent (PanVera Corp., Madison, WI) to assess the expression level and also the integrity of the expressed protein by ELISA and western blot analysis.

Methods for derivation of CHO cells stably expressing the SARS viral antigens of the invention comprise the steps of transfection and primary screening with selective medium. Optionally, these steps are followed by subcloning to assure purity of cell lines. Cell culture supernatants can be assayed using an antigen capture ELISA to quantify expression levels at all stages of selection and amplification.

For full-length Spike expression constructs, methanol fixed cells can be screened for internal expression by immunofluorescent staining using a rabbit anti-SARS antibody. Successive measurements at the T75-flask stage of expansion can be employed to assure stability of expression levels. The molecular mass and integrity of the expressed proteins can be checked by PAGE both under native and reducing and denaturing conditions, followed by immunoprobing.

In one embodiment, the pCMV3 vectors expressing SARS-CoV Spike proteins in either full-length or truncated forms is introduced into CHOK-1 cells using the Trans-LT-1 reagent. On day one, 1×10^6 cells are plated on 100 mm dishes in non-selective F12 media + 10% Fetal Bovine Serum + 4 mM Glutamine. On day two, the cells are transfected with a DNA:LT-1 mixture and the media then replaced with complete F12 media. Twenty-four to forty-eight hours later depending on the cell density, each 100 mm dish is split to 4-6 100 mm dishes. The medium is changed to complete selective media containing Geneticin (neomycin) at 500 μg/ml. All bovine serum used in these procedures is from TSE-free sources that meet current FDA standards. Twenty-four hours later the medium is changed to complete selective medium plus

500 ug/ml neomycin. Ten to fourteen days later, individual colonies are picked and transferred to 96 well plates and cultured in complete selective medium but without G418. When approximately 80% of the wells are confluent, twenty-four hour supernatants are screened by spike capture ELISA positive clones are transferred to twenty-four well plates. For the initial expression of full length Spike protein, methanol fixed cells will be screened by immunoflourescent staining using a rabbit anti-SARS antibody. After the low expressing cell lines have been eliminated and there are less than 20-30 cell lines, capture ELISA and westerns will be used to determine the expression level after cell lysis. A portion of each cell line will be pelleted, weighed and lysed in 1% triton lysis buffer containing MOPS, NaCl and $MgCl_2$ at the same ratio of cell weight to lysis buffer. After lysis the supernatant is collected and expression level is determined. Three to four clones producing the highest levels of spike protein in correct structure and conformation will be grown in three-liter bioreactors for expansion and adaptation to low serum suspension culture conditions for scale-up.

The antigen capture ELISA assay for the SARS spike protein can be performed as described in the art. A brief description of this assay follows. 96 well flat-bottom plates (Corning, Corning, NY) are coated with 250ng per well of purified immunoglobulin obtained from rabbit sera that were immunized with inactivated SARS virus. Between steps, the plates are washed in a buffer containing 16%NaCl and 1% Triton X100. 100μL of supernatant or lysate samples (diluted in a buffer containing 100mM $NaPO_4$, 0.1% Casein, 1mM EDTA, 1% Triton X100, 0.5M NaCl and 0.01% Thiomersal, pH 7.5) are added and incubated for 2 hours at 37°C. Bound antigen is reacted against pooled SARS+ve serum or high affinity monoclonal antibody either human or mouse against SARS spike protein (1 hour incubation, 37°C) and detected using appropriate species-specific peroxidase conjugated second antibody (30 minute incubation at 37°C; TAGO, Burlingame, CA). The plates are developed for 15 minutes at room temperature using TMB substrate (Pierce, Rockford, IL) and the reaction stopped using 4N phosphoric acid. The plates are read at a wavelength of 450nm and the concentration of protein per ml sample is derived from a standard curve (OD *vs.* protein concentration) based on serial dilutions of a known concentration of recombinant spike protein.

The immunoprobing analysis can also be performed following the standard methods described elsewhere in the art. A brief description follows. 10-20 μl of the sample is analyzed on 4-20% SDS PAGE under non-reducing/ denaturing conditions with mild heating. The gels are run for 1.5-2.0 hours at 100V constant voltage. The proteins are then transferred onto nitrocellulose membranes (Millipore, Bedford, MA) for 45 min using the semidry western transfer system (BioRad, Hercules, CA) following the manufacturer's instructions. The membrane is then reacted against polyclonal anti-spike rabbit serum, followed by anti-rabbit Ig

conjugated to Alexa 688 (Molecular Probes, Oregon). The blots are scanned using an infrared imaging system (LI-Cor, Inc., Lincoln, Nebraska).

The highest expressing candidate cell lines can be screened for spike protein expression and stability in small-scale (3 liter) suspension cultures. The candidate clone can be further evaluated for level of expression as well as integrity of expressed protein after amplification, and subsequently tested for expression stability in the absence of selection. The selected clones can also be tested for maintenance of the DNA sequence integrity of the integrated SARS spike protein gene. To quickly monitor the expression levels in small flask (T25 or T75) and in the three liter evaluation cultures, a lectin-based process (Gluvanthus Nivalis lectin) may be used to isolate SARS spike protein to a degree of purity that allows semi-quantitation and characterization of the protein in CHO supernatant. For full-length spike protein, it will be obtained from triton X-100 detergent extracted cells. Full-length Spike protein will be then captured on GNA lectin, followed by hydroxyapatite and SP chromatograph. Eluted protein is then characterized by: 1) polyacrylamide gel electrophoresis (PAGE) and Coomassie staining, 2) Immunoprobing with anti-SARS rabbit sera, 3) structural characterization using size exclusion chromatography (SEC), as well as mass spec analysis using MALDI-TOF.

Routes and methods of immunization of the vaccines of the invention are discussed in more detail in a section below. Examples 7 to 9 illustrate sample immunization protocols for the recombinant spike proteins.

Vaccine testing

Prior to human administration, it is normal to test vaccines in animal models. A mouse model of SARS coronavirus infection is known (Subbarao *et al.* (2004) *J Virol* 78:3572-77), and other animals that may be used as models of infection and/or disease include ferrets and monkeys. Thus the invention provides a non-human animal that is infected by the SARS coronavirus, wherein the animal is preferably a ferret or a primate (*e.g.* a monkey or a macaque). The animal may be gnotobiotic. The animal is preferably not a cat (*Felis domesticus*). The animal may or may not display SARS disease symptoms *e.g.* ferrets (*Mustela furo*) show prominent pulmonary pathology after infection. See: Martina *et al.* (2003) *Nature* 425:915.

E. Polynucleotides encoding the SARS Antigens of the Invention

The invention includes polynucleotides encoding for the SARS antigens of the invention. In addition, the invention includes polynucleotides which have been optimized for recombinant production (*e.g.* codon optimization) of the SARS antigens of the invention, including polynucleotides encoding for each of the SARS fusion constructs discussed above.

F. Viral vector or Viral Particle delivery of the SARS Antigens of the Invention

The antigens of the invention may be expressed *in vivo* or *in vitro* by polynucleotides encoding the antigens. Expression and delivery of the polynucleotides of the invention may be facilitated via viral vectors and/or viral particles.

Gene-based delivery systems derived from viruses, such as alphaviruses, are useful for the *ex vivo* and *in vivo* administration of heterologous genes, including one or more SARS genes, having therapeutic or prophylactic applications. These systems can also be used for the production of recombinant proteins derived from the SARS virus in cultured cells. Gene-based delivery systems of the invention include viral vectors (*e.g.*, adenovirus vector, poxvirus vector, alphavirus vector) and non-viral nucleic acid vectors (*e.g.*, DNA, RNA) encoding one or more SARS virus antigens. Polynucleotides encoding SARS virus antigen(s) are incorporated into the gene-based vaccines individually or in combination (*e.g.*, as bicistronic constructs).

1. Alphavirus

Alphaviruses are members of *Togaviridae* family and share common structural and replicative properties. Sindbis virus (SIN) is the prototype virus for the molecular study of other alphaviruses, and together with Venezuelan equine encephalitis virus (VEE) and Semliki Forest virus (SFV), are the most widely utilized alphaviruses being developed into expression vectors for heterologous genes (Schlesinger and Dubensky (1999) *Curr Opin. Biotechnol. 10*:434-439; Schlesinger (2001) Expert Opin. Biol. Ther. 1:177-91).

Alphaviruses possess a relatively small single-stranded RNA genome of positive polarity, which is approximately 12 kb in length, capped and polyadenylated. The RNA interacts with viral capsid protein monomers to form nucleocapsids, which in turn, are surrounded by a host cell-derived lipid envelope from which two viral glycoproteins, E1 and E2, protrude forming "spike" trimers of heterodimeric subunits. Two open reading frames (ORFs) encode as polyproteins the enzymatic nonstructural replicase proteins (5' ORF) and the virion structural proteins (3' ORF). The structural polyprotein is translated from a highly abundant subgenomic mRNA, which is transcribed from a strong internal alphavirus promoter (Strauss and Strauss (1994) *Microbiol. Rev.* 58:491-562). Replication of the genome occurs exclusively within the host cell cytoplasm as RNA.

The most common alphavirus expression vectors have exploited both the positive-stranded nature and modular organization of the RNA genome. These vectors, termed "replicons" due to their property of self-amplification, permit insertion of heterologous sequences in place of the structural polyprotein genes, while maintaining the 5'- and 3'-end *cis* replication signals, the nonstructural replicase genes, and the subgenomic junction region promoter (Xiong *et al.* (1989) *Science 243*:1188-1191; Liljestrom (1991) *Bio/Technology 9*:1356-1361). Chimeric alphavirus vectors (and particles) from sequences derived from divergent virus families have also been

described. (see, for example United States patent application serial number 09/236,140; see also, US Patents 5,789,245, 5,842,723, 5,789,245, 5,842,723, and 6,015,694; as well as WO 95/07994, WO 97/38087 and WO 99/18226). Co-owned International Publication WO 02/099035, published December 12, 2002 and incorporated by reference in its entirety herein, describes chimeric alphavirus molecules and modified alphavirus molecules having modified Biosafety Levels.

The absence of structural protein genes renders alphavirus replicon vectors defective, in that RNA amplification and high-level heterologous gene expression occurs within the target cell, but cell-to-cell spread of vector is not possible due to the inability to form progeny virions. Through the years, several synonymous terms have emerged that are used to describe alphavirus replicon particles. These terms include recombinant viral particle, recombinant alphavirus particle, alphavirus replicon particle and replicon particle. However, as used herein, these terms all refer to a virion-like unit containing an alphavirus-derived RNA vector replicon. Moreover, these terms may be referred to collectively as vectors, vector constructs or gene delivery vectors.

Packaging of replicon RNA into particles can be accomplished by introducing the replicon RNA into permissive cells (*e.g.*, RNA or DNA transfection, or particle infection) that also contain one or more structural protein expression cassettes or "defective helper" constructs encoding the alphavirus structural proteins. These structural protein encoding constructs may themselves be introduced into the cells by transfection of either RNA or DNA, and most commonly retain the native alphavirus subgenomic promoter, as well as 5'- and 3'-end *cis* signals for co-amplification with the replicon, but are devoid of any replicase genes and the RNA packaging signal (Liljestrom (1991) *Bio/Technology 9*:1356-1361; Pushko *et al.* (1997) *Virology 239*:389-401; Polo *et al.* (1999) *PNAS 96*:4598-4603). Permanent cell lines that are stable transformed with constructs expressing the alphavirus structural proteins (*e.g.*, packaging cell lines) offer a means to avoid transient transfection production methods (Polo *et al.* (1999) *PNAS 96*:4598-4603).

The present invention includes compositions and methods for the production of replication defective viral vector particles (*e.g.*, alphavirus replicon particles) for use in the *ex vivo* and *in vivo* administration of heterologous genes encoding proteins having therapeutic or prophylactic application, including genes encoding for one or more SARS viral antigens.

In one aspect, the invention includes a method of producing replication defective viral vector particles (*e.g.*, alphavirus replicon particles) comprising the steps of introducing at least one nucleic acid molecule comprising a viral vector (*e.g.*, alphavirus replicon RNA) into immortalized cells of the present invention, under conditions that allow for complementation of the viral vector (*e.g.*, alphavirus replicon RNA) and production of viral vector particles (*e.g.*, alphavirus replicon particles), and isolating the viral vector particles from the cells or cell culture

supernatants. In certain embodiments, the immortalized cells are grown in suspension, for example PERC.6 cells. In other embodiments, the methods are performed in large-scale volumes, for example, liter volumes or greater, such as for example in roller bottles, large flasks, Nunc Cell Factories, Corning Cell Cubes, fermentation vessels, etc).

In certain embodiments, the viral vector is an alphavirus replicon RNA that requires complementation by providing one or more alphavirus structural proteins in trans, within the immortalized cell. In such instances, the methods of complementation to produce alphavirus replicon particles may involve the introduction of one or more nucleic acids (*e.g.*, RNA, DNA) encoding said alphavirus structural protein(s) (*e.g.*, capsid and/or envelope glycoproteins) into the immortalized cells, either transiently or stably, and either concurrent with or prior to the introduction of the alphavirus replicon RNA. In certain embodiments, the alphavirus replicon RNA is introduced into the cell by transfection an in vitro transcribed RNA. In other embodiments, the alphavirus replicon RNA is introduced into the cell by transfection of a DNA (*e.g.*, ELVIS), which is capable of transcribing within the cell, the replicon RNA. In yet other embodiments, the alphavirus replicon RNA is introduced into the cell by infection with a seed stock of alphavirus replicon particles. In certain embodiments, the nucleic acids encoding said alphavirus structural protein(s) are defective helper RNA or are DNA that can transcribe within the cell defective helper RNAs.

As discussed herein, "alphavirus RNA replicon vector", "RNA replicon vector", "replicon vector" or "replicon" refers to an RNA molecule that is capable of directing its own amplification or self-replication *in vivo*, within a target cell. To direct its own amplification, the RNA molecule should encode the polymerase(s) necessary to catalyze RNA amplification (*e.g.*, alphavirus nonstructural proteins nsP1, nsP2, nsP3, nsP4) and also contain *cis* RNA sequences required for replication which are recognized and utilized by the encoded polymerase(s). An alphavirus RNA vector replicon should contain the following ordered elements: 5' viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred to as 5' CSE, or 5' *cis* replication sequence, or 5' viral sequences required in *cis* for replication, or 5' sequence which is capable of initiating transcription of an alphavirus), sequences which, when expressed, code for biologically active alphavirus nonstructural proteins (*e.g.*, nsP1, nsP2, nsP3, nsP4), and 3' viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred as 3' CSE, or 3' viral sequences required in *cis* for replication, or an alphavirus RNA polymerase recognition sequence). The alphavirus RNA vector replicon also should contain a means to express one or more heterologous sequence(s), such as for example, an IRES or a viral (*e.g.*, alphaviral) subgenomic promoter (*e.g.*, junction region promoter) which may, in certain embodiments, be modified in order to increase or reduce viral transcription of the subgenomic fragment, or to decrease homology with

defective helper or structural protein expression cassettes, and one or more heterologous sequence(s) to be expressed. Preferably the heterologous sequence(s) comprises a protein-encoding gene, which is the 3' proximal gene within the vector replicon. And preferably the replicon further comprises a polyadenylate tract.

As discussed herein, "recombinant Alphavirus Particle", "alphavirus replicon particle" and "replicon particle" refers to a virion-like unit containing an alphavirus RNA vector replicon. Generally, the recombinant alphavirus particle comprises one or more alphavirus structural proteins, a lipid envelope and an RNA vector replicon. Preferably, the recombinant alphavirus particle contains a nucleocapsid structure that is contained within a host cell-derived lipid bilayer, such as a plasma membrane, in which one or more alphaviral envelope glycoproteins (*e.g.*, E2, E1) are embedded. The particle may also contain other components (*e.g.*, targeting elements such as biotin, other viral structural proteins or portions thereof, hybrid envelopes, or other receptor binding ligands), which direct the tropism of the particle from which the alphavirus was derived. Generally the interaction between alphavirus RNA and structural protein(s) necessary to efficiently form a replicon particle or nucleocapsid may be an RNA-protein interaction between a capsid protein and a packaging signal or packaging sequence contained within the RNA.

"Alphavirus packaging cell line" refers to a cell which contains one or more alphavirus structural protein expression cassettes and which produces recombinant alphavirus particles (replicon particles) after introduction of an alphavirus RNA vector replicon, eukaryotic layered vector initiation system, or recombinant alphavirus particle. The parental cell may be of mammalian or non-mammalian origin. Within preferred embodiments, the packaging cell line is stably transformed with the structural protein expression cassette(s).

"Defective helper RNA" refers to an RNA molecule that is capable of being amplified and expressing one or more alphavirus structural proteins within a eukaryotic cell, when that cell also contains functional alphavirus nonstructural "replicase" proteins. The alphavirus nonstructural proteins may be expressed within the cell by an alphavirus RNA replicon vector or other means. To permit amplification and structural protein expression, mediated by alphavirus nonstructural proteins, the defective helper RNA molecule should contain 5'-end and 3'-end RNA sequences required for amplification, which are recognized and utilized by the nonstructural proteins, as well as a means to express one or more alphavirus structural proteins. Thus, an alphavirus defective helper RNA should contain the following ordered elements: 5' viral or cellular sequences required for RNA amplification by alphavirus nonstructural proteins (also referred to elsewhere as 5' CSE, or 5' *cis* replication sequence, or 5' viral sequences required in *cis* for replication, or 5' sequence which is capable of initiating transcription of an alphavirus), a means to express one or more alphavirus structural proteins, gene sequence(s) which, when expressed,

codes for one or more alphavirus structural proteins (*e.g.*, C, E2, E1), 3' viral or cellular sequences required for amplification by alphavirus nonstructural proteins (also referred to as 3' CSE, or 3' viral sequences required in *cis* for replication, or an alphavirus RNA polymerase recognition sequence), and a preferably a polyadenylate tract. Generally, the defective helper RNA should not itself encode or express in their entirety all four alphavirus nonstructural proteins (nsP1, nsP2, nsP3, nsP4), but may encode or express a subset of these proteins or portions thereof, or contain sequence(s) derived from one or more nonstructural protein genes, but which by the nature of their inclusion in the defective helper do not express nonstructural protein(s) or portions thereof. As a means to express alphavirus structural protein(s), the defective helper RNA may contain a viral (*e.g.*, alphaviral) subgenomic promoter which may, in certain embodiments, be modified to modulate transcription of the subgenomic fragment, or to decrease homology with replicon RNA, or alternatively some other means to effect expression of the alphavirus structural protein (*e.g.*, internal ribosome entry site, ribosomal readthrough element). Preferably an alphavirus structural protein gene is the 3' proximal gene within the defective helper. In addition, it is also preferable that the defective helper RNA does not contain sequences that facilitate RNA-protein interactions with alphavirus structural protein(s) and packaging into nucleocapsids, virion-like particles or alphavirus replicon particles. A defective helper RNA is one specific embodiment of an alphavirus structural protein expression cassette.

Alphavirus for use in the invention may be grown in any one of the cell lines discussed above as suitable for the SARS virus.

Alphavirus replicon particles may be produced according to the present invention by using the above cell lines (*e.g.*, immortalized cell lines) and a variety of published and accepted alphavirus vector methodologies. Such methodologies include, for example, transient packaging approaches, such as the co-transfection of *in vitro* transcribed replicon and defective helper RNA(s) (Liljestrom, *Bio/Technology 9*:1356-1361, 1991; Bredenbeek *et al.*, *J. Virol. 67*:6439-6446, 1993; Frolov *et al.*, *J. Virol. 71*:2819-2829, 1997; Pushko *et al.*, *Virology 239*:389-401, 1997; US Patents 5,789,245 and 5,842,723) or co-transfection of plasmid DNA-based replicon and defective helper construct(s) (Dubensky *et al.*, *J. Virol. 70*:508-519, 1996), as well as introduction of alphavirus structural protein expression cassettes (*e.g.*, DNA-based defective helper) into immortalized cell lines of the present invention to create stable packaging cell lines (PCL) (Polo *et al.*, *PNAS 96*:4598-4603, 1999; US Patents 5,789,245, 5,842,723, 6,015,694; WO 97/38087, WO 99/18226, WO 00/61772, and WO 00/39318). Stable packaging cell lines may then be utilized for alphavirus replicon particle production. The PCL may be transfected with *in vitro* transcribed alphavirus replicon RNA, transfected with a plasmid DNA-based replicon (*e.g.*, ELVIS vector), or infected with a seed stock of alphavirus replicon particles, and then incubated under conditions and for a time sufficient to produce progeny alphavirus replicon particles in the

culture supernatant. In addition, progeny replicon particles can subsequently be passaged in additional cultures of naïve PCL by infection, resulting in further expansion and commercial scale preparations. Importantly, by using defective helper RNA or stable PCL based on the "split" structural gene configuration, these replicon particle stocks may be produced free from detectable contaminating RCV.

Following harvest, crude culture supernatants containing the chimeric alphavirus replicon particles may be clarified by passing the harvest through a filter (*e.g.*, 0.2 uM, 0.45 uM, 0.65 uM, 0.8 uM pore size). Optionally, the crude supernatants may be subjected to low speed centrifugation prior to filtration to remove large cell debris. Within one embodiment, an endonuclease (*e.g.*, Benzonase, Sigma #E8263) is added to the preparation of alphavirus replicon particles before or after a chromatographic purification step to digest exogenous nucleic acid. Further, the preparation may be concentrated prior to purification using one of any widely known methods (*e.g.*, tangential flow filtration). Crude or clarified alphavirus replicon particles may be concentrated and purified by chromatographic techniques (*e.g.*, ion exchange chromatography, size exclusion chromatography, hydrophobic interaction chromatography, affinity chromatography), such as those described in WO01/92552, incorporated by reference in its entirety herein. Two or more such purification methods may be performed sequentially.

EXAMPLE OF ALPHAVIRUS REPLICON PARTICLES ENCODING SARS VIRUS SPIKE (S) ANTIGEN

The invention includes compositions and methods for the production of replication defective viral vector particles (*e.g.*, alphavirus replicon particles) for use in the ex vivo and in vivo administration of heterologous genes encoding proteins having therapeutic or prophylactic application, including genes encoding for one or more SARS viral antigens.

The following example illustrates a method of preparing alphavirus replicon particles encoding SARS virus spike (s) antigen.

The SARS virus spike gene can be incorporated into alphavirus replicon particles derived from a variety of alphavirus, such as Sindbis virus, Semliki Forest virus (US 5739026), Venezuelan equine encephalitis virus (US 6531135), and replicon particle chimeras derived from more than one alphavirus (US 6376236, WO 02/99035). In addition, the SARS virus spike gene can be incorporated in its entirety (encoding full-length spike protein) or in a modified form that includes, for example, sequence deletions or truncations, such that the encoded a spike protein is of less than full-length (*e.g.*, C-terminal truncation of one or more (*e.g.* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 *etc.*) amino acids, deleted of transmembrane region and cytoplasmic tail).

For example, the spike gene may be cloned as a full-length gene into the VCR-chim2.1 vector (WO 02/99035) by standard RT-PCR conditions or by standard subcloning from one of the other plasmids described herein, using commercially available restriction endonucleases. For

the reverse transcription step in standard RT-PCR, the Superscript pre-amplification kit (Invitrogen™) and the primer SEQ ID NO: 7325 (sp-RT-R) are used:

For the amplification step, the cDNA polymerase advantage kit (Clonetech) and two primers Sp-F-BbvCI (SEQ ID NO: 7326) and Sp-R-NotI (SEQ ID NO: 7327) are used:

The forward primer is designed to contain the ccacc sequence (Kozak, 1991 *JBC* 19867-70) in front of the ATG codon to optimize translation efficiency of the spike gene. Also, the forward primer contains the BbvCI restriction site and the reverse primer contains the NotI restriction site for subsequent cloning of the PCR amplified gene.

The PCR product is purified using the QIAquick Nucleotide Removal kit (QIAgen), digested with BbvCI and NotI, gel purified with QIAquick Gel Extraction kit (QIAgen), and ligated to plasmid VCR-Chim2.1 pre-digested with the same enzymes. Clones containing the SARS spike sequence are verified by sequencing and the new construct is called VCR-Chim2.1-SARSspike.

To generate VEErep/SINenv-SARSspike replicon particles the plasmids VCR-Chim2.1-SARSspike, VCR-DH-Scap (WO 02/99035), and VCR-DH-Sglydl160 (WO 02/99035) are linearized with the restriction enzyme PmeI and used for *in vitro* transcription as described previously (Polo *et al.* 1999, PNAS 96: 4598-603; WO02/99035). The transcripts are co-transfected into BHK cells as previously described (Polo *et al.*, 1999, *ibid.*; WO02/99035). The transfected cells are incubated at 34 ^{0}C, the supernatants collected at 20 and 30 hrs post-electroporation, clarified by centrifugation, and purified by chromatography as previously described (WO 01/92552).

Expression of the SARS spike protein from the replicon particle vector is verified by infecting BHK cells overnight with purified VEErep/SINenv-SARSspike or VEErep/SINenv-GFP (WO 02/99035) replicon particles. In addition, BHK cells also were transfected in parallel with in vitro transcribed VCR-Chim2.1-SARSspike replicon RNA. At 16 hrs post-infection and transfection cells are lysed and a sample of the lysate analyzed by western blot using an antibody that recognizes SARS virus spike protein. The proteins on the gel are stained or transferred to a membrane for Western blot analysis with sera from convalescent patients or alternatively murine or rabbit antisera generated against SARS virus. VEErep/SINenv-SARSspike replicon particles are administered to the vaccine recipient (*e.g.*, rodent, non-human primate, human) as described elsewhere in the present invention.

Figure 67 shows data from western blot analysis performed under non-reducing conditions, using a SARS virus specific rabbit polyclonal antisera. The western data demonstrate that not only is SARS spike protein expressed in cells infected with alphavirus replicon particles or transfected with replicon RNA, but the predominant form of spike is that of a homotrimer (Fig.67A). Similar homotrimeric association of the spike protein was observed in western blots

of SARS virions purified from SARS virus infected VERO cell supernatants, and this homotrimer is heat labile, as indicated by the dissociation into monomeric forms at 80°C and 100°C (Fig.67B).

To further characterize SARS Spike protein expression and processing following expression from alphavirus replicon vectors, BHK-21 cells were infected with alphavirus replicon particles expressing the full-length Spike. At 6 hr post-infection with an MOI of 5, infected cells were labeled for 1 hr with L-[^{35}S]methionine/cysteine and chased for the indicated time. The [^{35}S]-labeled spike protein was immunoprecipitated by anti-SARS rabbit serum and digested with Endo-H. Both digested and undigested proteins were analysed by 4% polyacrylamide-SDS PAGE under reducing conditions. As shown in Figure 55, the full-length spike protein is synthesized as an Endo-H sensitive high mannose glycoprotein (gp170, an ER form) that undergoes modification to an Endo-H resistant glycoprotein with complex oligosaccharides (gp180, a Golgi form). The conversion of gp170 into the gp180 form takes place within 2 hr.

Alphavirus replicon particles expressing one or more SARS proteins (e.g., VEErep/SINenv-SARSspike replicon particles) are administered to the vaccine recipient in order to induce a SARS specific immune response (*e.g.*, rodent, ferret, non-human primate, human) as described elsewhere in the present invention. Immunization may be performed through a variety of routes, including for example, intramuscular, subcutaneous, intradermal, and intranasal. In additon, the alphavirus replicon particles may be used alone or in combination (*e.g.*, "prime-boost") with other vaccine approaches of the present invention, or alternatively the alphavirus replicon particles may co-express antigen from other respiratory pathogens or be co-administered in combination with alphavirus replicon particles expressing antigens from other respiratory pathogens (*e.g.*, influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus). For example, the induction of anti-spike protein antibodies in animals immunized IM with VEErep/SINenv-SARSspike replicon particles was demonstrated in mice (Figure 68). These mouse studies also included addtional vaccine groups for comparison, including the inactivated SARS virus and recombinant truncated spike protein vaccines describe elsewhere herein, as well as plasmid DNA used as a prime, followed by alphavirus replicon particles as a boost. The data clearly show very potent immune responses for all vaccine groups, including the alphavirus replicon particle group. It should be noted that the level of antibody induced by the inactivated SARS virus vaccine used in these experiments has been shown to be protective in a SARS virus animal challenge model.

Similarly, genes encoding other SARS virus antigens (*e.g.*, nucleocapsid protein, membrane glycoprotein) are cloned into alphavirus replicon vectors, either individually or in

combination, to generate alphavirus replicon particles according to the teachings of the present invention and using standard molecular biology techniques..

EXAMPLE OF ALPHAVIRUS-BASED PLASMID DNA EXPRESSING SARS VIRUS SPIKE (S)

The invention includes preparation of plasmid DNA expressing a SARS virus antigen for prophylactic or therapeutic immunization against SARS virus infection. In one embodiment, the SARS viral antigen is a spike (S) protein. In one embodiment, the plasmid DNA is alphavirus-based.

The following example illustrates one method for preparing an alphavirus-based plasmid DNA expressing SARS virus spike (S).

SARS spike gene can be delivered using any of the alphavirus-based plasmid DNA replicons such as ELVS (Dubensky et al, 1996 J Virol. 70: 508-19), SINCP (WO 01/81609), or VCP (PCT WO 02/99035).

For example, the SARS spike gene is cloned into SINCP using the standard RT-PCR techniques. The oligo Sp-RT-R is used for the reverse transcription step with the Superscript pre-amplification kit (Invitrogen). For the amplification step, the cDNA polymerase advantage kit (Clonetech) with the Sp-R-NotI and Sp-F-XhoI (SEQ ID NO: 7328) primers is used.

The Sp-F-XhoI primer was designed to contain the ccacc sequence in front of the ATG codon to optimize translation efficiency (Kozak 1991, ibid) of the spike gene. Also, the primer contains the XhoI restriction site for the subsequent cloning of the PCR amplified gene.

The PCR product is purified using the QIAquick Nucleotide removal kit, digested with XhoI and NotI, gel purified with QIAquick Gel Extraction kit, and ligated to plasmid SINCP pre-digested with the same enzymes. Clones containing the SARS spike sequence are verified by sequencing and the new construct is called SINCP-SARSspike.

Expression of the SARS spike gene is verified by transient transfection of BHK cells with 2μg of either plasmid DNA SINCP-SARSspike or SINCP pre-incubated for 5 minutes with 5 μl of TransIT Polyamine reagent (Mirrus) in low serum medium Optimem (Life Technologies). At 48 hrs pos-transfection cells are lysed and a sample of the lysate is run on 8% SDS-PAGE. The proteins on the gel are either stained or transferred to a membrane for Western blot analysis with sera from convalescent patients, or alternatively with sera from mouse or rabbits.

SINCP-SARSspike plasmid replicon is administered to the vaccine recipient (*e.g.*, rodent, non-human primate, human) as a formulated or unformulated plasmid vaccine, alone or in combination (*e.g.*, "prime-boost") with other vaccines of the present invention, as described elsewhere herein.

Similarly, genes encoding other SARS virus antigens (*e.g.*, nucleocapsid protein, membrane glycoprotein) are cloned into alphavirus plasmid replicon vectors.

2. Plasmid Expression Vectors

EXAMPLE OF PLASMID DNA EXPRESSING SARS VIRUS SPIKE (S)

The following example illustrates a method for preparing plasmid DNA expressing SARS virus spike (s).

The SARS virus spike antigen also may be delivered using other plasmid DNA expression vectors (sometimes referred to as "conventional" DNA vaccines), based on a polymerase II promoter, such as, for example, a CMV promoter. A DNA vaccine of the spike antigen gene induces an antibody response in mice (Zhao *et al.* (2004) *Acta Biochim et Biophysica Sinica* 36:37-41), and has been found to induce viral neutralization and protective immunity in mice (Yang *et al.* (2004) *Nature* 428:561-564), particularly when truncated at the C-terminus.

For example, the SARS spike gene is cloned into pCMVKm2 (Zur Megede *et al.*, J.Virol., 74:2628-2635, 2000; SEQ ID NO: 9923) using standard RT-PCR techniques. The oligo Sp-RT-R is used for the reverse transcription step with the Superscript pre-amplification kit (Invitrogen). For the amplification step, the cDNA polymerase advantage kit (Clonetech) is used with primers Sp-F-EcoRI (SEQ ID NO: 7329) and Sp-R-XbaI (SEQ ID NO: 7330).

The forward primer was designed to contain the CCACC (SEQ ID NO: 7331) sequence in front of the ATG codon to optimize translation efficiency (Kozak 1991, ibid.) of the spike gene. Also, the forward primer contains the EcoRI restriction site and the reverse primer contains the XbaI restriction site for the subsequent cloning of the PCR amplified gene.

The PCR product is purified using the QIAquick Nucleotide Removal kit, digested with XhoI and NotI, gel purified with QIAquick Gel Extraction kit, and ligated to plasmid pCMVKm2 pre-digested with the same enzymes. Clones containing the SARS spike sequence are verified by sequencing and the new construct is called pCMVKm2-SARSspike.

Expression of the SARS spike gene is verified by transient transfection of BHK or 293 cells with 2μg of either plasmid DNA pCMVKm2-SARSspike or pCMVKm2 pre-incubated for 5 minutes with 5 μl of TransIT Polyamine reagent (Mirrus) in low serum medium Optimem (Life Technologies). At 48 hrs pos-transfection cells are lysed and a sample of the lysate is run on 8 % SDS-PAGE. The proteins on the gel are either stained or transferred to a membrane for Western blot analysis with sera from convalescent patients, or alternatively using mouse or rabbit antisera.

Plasmid pCMVKm2-SARSspike is administered to the vaccine recipient (*e.g.*, rodent, non-human primate, human) as a formulated or unformulated plasmid vaccine, as described elsewhere in the present invention.

Similarly, genes encoding other SARS virus antigens (*e.g.*, nucleocapsid protein, membrane glycoprotein) are cloned into plasmid expression vectors

3. Virus-Like Particles comprising SARS antigens

The SARS viral antigens of the invention may be formulated into Virus Like Particles ("VLPs"). The invention thus includes virus-like particles (or VLPs) comprising one or more SARS viral antigens. Preferably, the VLPs comprise one or more SARS viral antigens selected from the group consisting of Spike (S), nucleocapsid (N), membrane (M) and envelope (E). Preferably, the VLPs comprise at least M and E.

The VLPs of the invention comprise at least one particle-forming polypeptide. Said particle-forming polypeptide is preferably selected from a Coronavirus structural protein. In one embodiment, the particle-forming polypeptide is selected from one or more SARS viral antigens. In another embodiment, the particle-forming polypeptide is selected from the structural protein of a non-SARS Coronavirus, such as, for example, Mouse Hepatitis Virus.

VLPs can be formed when viral structural proteins are expressed in eukaryotic or prokaryotic expression systems. Upon expression, the structural proteins self-assemble to form particles. Alternatively, viral structural proteins may be isolated from whole virus and formulated with phospholipids. Such viral structural proteins are referred to herein as "particle-forming polypeptides". VLPs are not infectious because no viral genome is present, however, these non-replicating, virus capsids mimic the structure of native virions.

Due to their structure, VLPs can display a large number of antigenic sites on their surface (similar to a native virus). VLPs offer an advantage to live or attenuated vaccines in that they are much safer to both produce and administer, since they are not infectious. VLPs have been shown to induce both neutralizing antibodies as well as T-cell responses and can be presented by both class I and II MHC pathways.

Previous work creating VLPs from coronavirus indicates that E and M proteins along may be sufficient for coronavirus VLP formation. *See* Fischer *et al.*, *J. Virol.* (1998) 72:7885-7894 and Vennema *et al. EMBO J.* (1996) 15:2020-2028.

Chimeric VLPs comprising particle-forming polypeptides or portions thereof from non-SARS Coronaviruses are also included in the invention. Such particle-forming polypeptides may comprise a full length polypeptide from a non-SARS Coronavirus. Alternatively, a particle-forming fragment may be used.

In one embodiment, a fragment of a non-SARS particle-forming polypeptide and a fragment of a SARS viral antigen are fused together. For instance, such chimeric polypeptides may comprise the the endodomain and transmembrane domain of a non-SARS particle-forming polypeptide and the ectodomain of a SARS viral antigen. In one example, the VLPs of the invention comprise a chimeric spike protein comprising an endodomain and transmembrane domain of the spike protein of Mouse Hepatitis Virus (MHV) and the chimeric spike protein further comprises the ectodomain of the SARS spike protein. Such VLPs may further comprise

Coronavirus M and E proteins. Said M and E proteins may be selected from any coronavirus, including Mouse Hepatitis Virus (MHV) or SARS. Sample sequences of S, M and E proteins of MHV are included in the figures, *supra.*

Chimeric spike proteins derived from the ectodomain of feline infectious peritonitis virus (FIPV) spike protein fused to the endo and transmembrane domains of MHV spike protein have been previously disclosed. See WO 98/49195 and WO 02/092827. In these chimeric VLP structures, the capsid structure of the VLPs is formed by the M and E protein of MHV. The chimeric spike protein provides for the surface exposure of the ectodomain of the FIPV spike protein.

As used herein, the term "virus-like particle" or "VLP" refers to a non-replicating, empty virus shell. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an approrpirate expression system. Alternatively, viral structural proteins may be isolated from whole virus and formulated with phospholipids. Methods for producing particular VLPs are known in the art and discussed more fully below. The presence of VLPs in a composition can be detected using conventional techniques known in the art, such as by electron microscopy, x-ray crystallography, and the like. See, *e.g.*, Baker *et al.*, *Biophys. J.* (1991) 60:1445-1456; Hagensee *et al.*, *J. Virol.* (1994) 68:4503-4505. For example, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

The phrase "particle-forming polypeptide" includes a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletion, which has the ability to form VLPs under conditions that favor VLP formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore includes deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also includes deletions, addition and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP.

Preferred substitutions are those which are conservative in nature, *i.e.*, those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic: aspartate and glutamate; (2) basic: lysine, arginine, and histidine; (3) non-polar: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar: glycine, asparagine, glutamine,

cystine, serine, theronine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, an asparate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule, but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein, are therefore within the definition of the reference polypeptide.

The VLPs of the invention can be formed from any viral protein, particle-forming polypeptide derived from the viral protein, or combination of viral proteins or fragments thereof, that have the capability of forming particles under appropriate conditions. The requirements for the particle-forming viral proteins are that if the particle is formed in the cytoplasm of the host cell, the protein must be sufficiently stable in the host cell in which it is expressed such that formation of virus-like structures will result, and that the polypeptide will automatically assemble into a virus-like structure in the cell of the recombinant expression system used. If the protein is secreted into culture media, conditions can be adjusted such that VLPs will form. Furthermore, the particle-forming protein should not be cytotoxic in the expression host and should not be able to replicate in the host in which the VLP will be used.

Preferred particle-forming polypeptides include coronavirus M and E proteins, preferably SARS M and E proteins.

Methods and suitable conditions for forming particles from a wide variety of viral proteins are known in the art. VLPs have been produced, for example from proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Niikura *et al.*, *Virology* (2002) 293:273-280; Lenz *et al.*, *J. Immunology* (2001) 5246-5355; Pinto, *et al.*, *J. Infectious Diseases* (2003) 188:327-338; and Gerber *et al.*, *J. Virology* (2001) 75(10):4752-4760.

As explained above, VLPs can spontaneously form when the particle-forming polypeptide of interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs for use in the present invention may be prepared using recombinant techniques, well known in the art. In this regard, genes encoding the particle-forming polypeptide in question can be isolated from DNA libraries or directly from cells and tissues containing the same, using known techniques. The genes encoding the particle-forming polypeptides can also be produced synthetically, based on the known sequences. The nucleotide sequence can be designed with the appropriate codons for

the particular amino sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed (*e.g.* human codons for human DNA vaccines). The complete sequence is generally assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See., *e.g.*, Edge, *Nature* (1981) 292:756; Nambair *et al. Science* (1984) 223:1299; Jay *et al.*, *J. Biol. Chem.* (1984) 259:6311.

Once the coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be cloned into any suitable vector or replicon for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Sambrook *et al.* The vector is then used to transform an appropriate host cell. Suitable expression systems include, but are not limited to, bacterial, mammalian, bacuolvirus/insect, vaccinia, Semliki Forest virus (SFV), yeast, and Xenopus expression systems, well known in the art.

A number of cell lines suitable for use as host cells for producing the VLPs of the invention are known in the art. Suitable mammalian cell lines include, but are not limited to, Chinese Hamster Ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), Madin-Darby bovine kidney ("MDBK") cells, as well as others. Mammalian sources of cells include, but are not limited to, human or non-human primate (*e.g.*, MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75), HUH, human embryonic kidney cells (293 cells, typically transformed by sheared adenovirus type 5 DNA), VERO cells from monkey kidneys (including, for example COS7 cells), horse, cow (*e.g.*, MDBK cells), sheep, dog (*e.g.*, MDCK cells from dog kidneys, ATCC CCL34 MDCK (NBL2) or MDCK 33016, deposit number DSM ACC 2219 as described in WO 97/37001), cat, and rodent (*e.g.*, hamster cells such as BHK21-F, HKCC cells, or Chinese hamster ovary cells (CHO cells)), and may be obtained from a wide variety of developmental stages, including for example, adult, neonatal, fetal, and embryo.

Bacterial hosts suitable for production of VLPs of the invention include *E. coli*, *Bacillus subtilis*, and *Streptoccocus spp.* Yeast hosts suitable for production of VLPs of the invention include *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells suitable for production of VLPs of the invention (*i.e.*, via baculovirus expression vectors) include *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodptera frugiperda*, and *Trichoplusia ni*.

Viral vectors can be used for the production of particles in eukaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additional,

vaccinia based infection/transfection systems, such as those as described in Tomei *et al., J. Virol* (1993) 67:4017-4026 and Selby *et al., J. Gen. Virol.* (1993) 74:1103-1113, can also be used to generate the VLPs of the invention. In this system, cells are first transfected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translation machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products.

Depending on the expression system and host selected, the VLPs are produced by growing host cells transformed by an expression vector under conditions whereby the particle-forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPs are formed intracellularly, the cells are then disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such methods are known the those of skill in the art and are described in, *e.g., Protein Purification Applications: A Practical Approach,* (E.L.V. Harris and S. Angal, Eds.; 1990).

The particles are then isolated using methods that preserve the integrity thereof, such as by gradient centrifugation, *e.g.*, cesium chloride (CsCl) and sucrose gradients, and the like (see, *e.g.*, Kirnbauer *et al., J. Virol.* (1993) 67:6929-6936), ion exchange chromatography (including anion exchange chromatography such as DMAE and TMAE), hydroxyapatitie chromatography (see WO 00/09671), hydrophobic interaction chromatography, gel filtration chromatography and other filtration methods such as nanometric filtration and ultrafiltration. Preferably at least one anion exchange step is performed during purification, and more preferably at least two anion exchange steps are used.

VLP formulations of the invention may be further processed by methods known in the art to disassemble the VLPs into smaller, protein containing moieties using a high concentration of reducing agent, followed by reassembly of the VLPs by either removal of the reducing agent or by addition of excess oxidant. The resulting reassembled VLPs may have improved homogeneity, stability and immunogenic properties. In addition, further therapeutic or prophylactic agents may be formulated into the VLPs upon reassembly. See McCarthy *et al., J. Virology* (1998) 72(1):32-41. See also WO 99/13056 and WO 01/42780. Reducing agents suitable for use in VLP disassembly include sulfhydryl reducing agents (such as glutathion, beta mercaptoethanol, dithiothreitol, dithioerythritol, cysteine, hydrogen sulfide and mixtures thereof) preferably contained in moderate to low ionic strength buffers. Sufficient exposure time of the VLPs to the reducing agent will be required to achieve a suitable amount of VLP disassembly.

Adjuvants may be added to the VLPs of the invention to enhance the immunogenicity of the SARS viral antigens. Antigens suitable for use with VLPs include those described, supra. For example, the VLPs of the invention may be adsorbed onto an aluminum adjuvant.

The VLPs of the invention may formulated to enhance their stability. Additional components which may enhance the stability of a VLP formulation include salts, buffers, non-ionic surfactants and other stabilizers such as polymeric polyanion stabilizers. See WO 00/45841.

The ionic strength of a solution comprising VLP particles may be maintained by the presence of salts. Almost any salt which can contribute to the control of the ionic strength may be used. Preferred salts which can be used to adjust ionic strength include physiologically acceptable salts such as NaCl, KCl, Na_2SO_4, $(NH_4)_2SO_4$, sodium phosphate and sodium citrate. Preferably, the salt component is present in concentrations of from about 0.10 M to 1 M. Very high concentrations are not preferred due to the practical limitations of parenteral injection of high salt concentrations. Instead, more moderate salt concentrations, such as more physiological concentrations of about 0.15M to about 0.5M with 0.15M-0.32M NaCl are preferred.

Buffers may also be used to enhance the stability of the VLP formulations of the invention. Preferably, the buffer optimizes the VLP stability while maintaining the pH range so that the vaccine formulation will not be irritating to the recipient. Buffers preferably maintain the pH of the vaccine formulation within a range of p/H 5.5-7.0, more preferably 6.0-6.5. Buffers suitable for vaccine formulations are known in the art and include, for example, histidine and imidazole. Preferably, the concentration of the buffer will range from about 2mM to about 100 mM, more preferably 5 mM to about 20 mM. Phosphate containing buffers are generally not preferred when the VLP is adsorbed or otherwise formulated with an aluminum compound.

Non-ionic surfactants may be used to enchance the stability of the VLP formulations of the invention. Surfactants suitable for use in vaccine formulations are known in the art and include, for example, polyoxyethylene sorbital fatty acid esters (Polysorbates) such as Polysorbate 80 (*e.g.*, TWEEN 80), Polysorbate 20 (*e.g.*, TWEEN 20), polyoxyethylene alkyl ethers (*e.g.*, Brij 35, Brij 58), as well as others, including Triton X-100, Triton X-114, NP-40, Span 85 and the Pluronic series of non-ionic surfactants (*e.g.*, Pluronic 121). The surfactant is preferably present in a concentration of from about 0.0005% to about 0.5% (wt/vol).

Polymeric polyanion stabilizers may also be used to enchance the stability of the VLP formulations of the invention. Suitable polymeric polyanionic stabilizers for use in the invention comprise either a single long chain or multiple cross linked chains; either type possessing multiple negative charges along the chains when in solution. Examples of suitable polyanionic polymers include proteins, polyanions, peptides and polynucelic acids. Specific examples include carboxymethyl cellulose, heparin, polyamino acids (such as poly(Glu), poly(Asp), and

Poly (Glu, Phe), oxidized glutathione, polynuceltodies, RNA, DNA and serum albumins. The concentration of the polmeric polyanion stabilizers is preferably from about 0.01% to about 0.5%, particularly about 0.05-0.1% (by weight).

G. Passive Immunization via Antibodies to the SARS Antigens of the Invention

The invention includes antibodies specific to the SARS antigens of the invention and methods of treatment or prevention of SARS virus related disease by administrating an effective amount of SARS antibodies to a mammalian subject. Antibodies specific the SARS antigens can be produced by one skilled in the art. Preferably, the antibodies are specific to the spike (S) protein of the SARS virus. Potent neutralization of the SARS coronavirus using a human monoclonal anti-spike antibody has been reported (Sui *et al.* (2004) *PNAS USA* 101:2536-2541). A IgG1 form of the monoclonal antibody showed a higher affinity (1.59 nM) than a scFv form (32.3 nM).

The antibodies of the invention are specific and selective to SARS antigens.

In one embodiment, the antibodies of the invention are generated by administering a SARS antigen to an animal. The method may also include isolating the antibodies from the animal.

The antibodies of the invention may be polyclonal or monoclonal antibody preparations, monospecific antisera, human antibodies, or may be hybrid or chimeric antibodies, such as humanized antibodies, altered antibodies (Fab')$_2$ fragments, F(ab) fragments, Fv fragments, single-domain antibodies, dimeric or trimeric antibody fragments or constructs, minibodies, or functional fragments thereof which bind to the antigen in question.

Antibodies are produced using techniques well known to those of skill in the art and disclosed in, for example, US Patent Nos. 4,011,308; 4,722,890; 4,016,043; 3,876,504; 3,770,380; and 4,372,745. For example, polyclonal antibodies are generated by immunizing a suitable animal, such as a mouse, rat, rabbit, sheep, or goat, with an antigen of interest. In order to enhance immunogenicity, the antigen can be linked to a carrier prior to immunization. Such carriers are well known to those of ordinary skill in the art. Immunization is generally performed by mixing or emulsifying the antigen in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). The animal is generally boosted 2-6 weeks later with one or more injections of the antigen in saline, preferably using Freund's incomplete adjuvant. Antibodies may also be generated by in vitro immunization, using methods known in the art. Polyclonal antiserum is then obtained from the immunized animal.

Monoclonal antibodies are generally prepared using the method of Kohler & Milstein (1975) *Nature* 256:495-497, or a modification thereof. Typically, a mouse or rat is immunized as described above. Rabbits may also be used. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into

single cells. If desired, the spleen cells may be screened (after removal of non-specifically adherent cells) by applying a cell suspension to a plate or well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected monoclonal antibody-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (*e.g.*, as ascites in mice).

Humanized and chimeric antibodies are also useful in the invention. Hybrid (chimeric) antibody molecules are generally discussed in Winter *et al.* (1991) *Nature* 349: 293-299 and US Patent No. 4,816,567. Humanized antibody molecules are generally discussed in Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyan *et al.* (1988) *Science* 239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994). One approach to engineering a humanized antibody involves cloning recombinant DNA containing the promoter, leader, and variable-region sequences from a mouse antibody gene and the constant-region exons from a human antibody gene to create a mouse-human chimera, a humanized antibody. See generally, Kuby, "Immunology, 3rd Edition", W.H. Freeman and Company, New York (1998) at page 136.

Antibody fragments which retain the ability to recognize a SARS antigen are also included within the scope of the invention. A number of antibody fragments are known in the art which comprise antigen-binding sites capable of exhibiting immunological binding properties of an intact antibody molecule. For example, functional antibody fragments can be produced by cleaving a constant region, not responsible for antigen binding, from the antibody molecule, using *e.g.*, pepsin, to produce $F(ab')_2$ fragments. These fragments will contain two antigen binding sites, but lack a portion of the constant region from each of the heavy chains. Similarly, if desired, Fab fragments, comprising a single antigen binding site, can be produced, *e.g.*, by digestion of polyclonal or monclonal antibodies with papain. Functional fragments, includnig only the variable regions of the heavy and light chains, can also be produced, using standard techniques such as recombinant production or preferential proteolytic cleavage of immunoglobulin molecules. These fragments are known as F_v. See, *e.g.*, Inbar *et al.* (1972) *Proc. Nat. Acad. Sci USA* 69:2659-2662; Hochman *et al.* (1976) *Biochem* 15:2706-2710; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096.

A single-chain Fv ("sFv" or scFv") polypeptide is a covalently linked V_H-V_L heterodimer which is expressed from a gene fusion including V_H-and V_L- encoding genes linked by a peptide-

encoding linker. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85:5879-5883. A number of methods have been described to discern and develop chemical strucutres (linkers) for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, US Patent Nos. 5,091,513; 5,132,405; and 4,946,778. The sFv molecules may be produced using methods described in thea rt. See, *e.g.*, Huston *et al.* (1988) *Proc. Nat. Acad. Sci USA* 85:5879-5338; US Patent Nos. 5,091,513; 5,132,405 and 4,946,778. Design criteria include determining the appropriate length to span the distance between the C-terminus of one chain and the N-terminus of the other, wherein the linker is generally formed from small hydrophilic amino acid residues that do not coil or form secondary structures. Such methods have been described in the art. See, *e.g.*, US Patent Nos. 5,091,513; 5,132,405 and 4,946,778. Suitable linkers generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility. Anti-spike scFv antibodies have been reported (Sui *et al.* (2004) *PNAS USA* 101:2536-2541).

"Mini-antibodies" or "minibodies" will also find use with the present invention. Minibodies are sFv polypeptide chains which include oligomerization domains at their C-termini, separated from the sFv by a hinge region. Pack *et al.*, (1992) *Biochem* 31:1579-1584. The oligomerization domain comprises self-associating α-helices, *e.g.*, leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to facilitate in vivo folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, *e.g.*, Pack *et al.*, (1992) *Biochem* 31:1579-1584; Cumber *et al.* (1992) *J. Immunology* 149B:120-126.

Non-conventional means can also be used to generate and identify the antibodies of the invention. For example, a phage display library can be screened for antibodies which bind to the SARS antigens of the invention. See generally, Siegel, "Recombinant Monoclonal Antibody Technology", *Transfus. Clin. Biol.* (2002) 9(1): 15-22; Sidhu, "Phage Display in Pharmaceutical Biotechnology", *Curr. Opin. Biotechnol.* (2000) 11(6):610-616; Sharon, *et al.*, "Recombinant Polyclonal Antibody Libraries", *Comb. Chem. High Throughput Screen* (2000) 3(3): 185-196; and Schmitz *et al.*, "Phage Display: A Molecular Tool for the Generation of Antibodies-Review", *Placenta*, (2000) 21 SupplA: S106-12.

The antibodies of the invention may also be generated by administering the polynucleotide sequence encoding for the SARS antigen into an animal. The SARS antigen is then expressed in vivo, and antibodies specific to the SARS antigen are generated *in vivo*. Methods for polynucleotide delivery of the SARS antigens of the invention are discussed in section 4 below.

The antibodies of the invention are preferably specific to the SARS virus.

H. Combinations of one or more of any of the above approaches in a vaccine

The compositions of the invention further comprise combinations of one or more of the compositions discussed above. For instance, the invention comprises a composition comprising an attenuated SARS virus and a subunit SARS viral antigen.

I. Combinations of SARS antigens and other Respiratory Virus Antigens

The invention further relates to vaccine formulations comprising one or more SARS virus antigens and one or more other respiratory virus antigens. Additional respiratory virus antigens suitable for use in the invention include antigens from influenza virus, human rhinovirus (HRV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, and rhinovirus. The additional respiratory virus antigen could also be from a coronavirus other than the SARS coronavirus, such as the NL63 human coronavirus (van der Hoek *et al.* (2004) *Nature Medicine* 10:368-373). Preferably, the additional respiratory virus antigen is an influenza viral antigen.

The invention may also comprise one or more bacterial or viral antigens in combination with the SARS viral antigen. Antigens may be used alone or in any combination. (See, *e.g.*, WO 02/00249 describing the use of combinations of bacterial antigens). The combinations may include multiple antigens from the same pathogen, multiple antigens from different pathogens or multiple antigens from the same and from different pathogens. Thus, bacterial, viral, and/or other antigens may be included in the same composition or may be administered to the same subject separately. It is generally preferred that combinations of antigens be used to raise an immune response be used in combinations.

Non-limiting examples of bacterial pathogens which may be used in the invention include diphtheria (*See, e.g.*, Chapter 3 of *Vaccines*, 1998, eds. Plotkin & Mortimer (ISBN 0-7216-1946-0), staphylococcus (*e.g.*, *Staphylococcus aureus* as described in Kuroda *et al.* (2001) *Lancet* 357:1225-1240), cholera, tuberculosis, *C. tetani,* also known as tetanus (*See, e.g.*, Chapter 4 of *Vaccines*, 1998, eds. Plotkin & Mortimer (ISBN 0-7216-1946-0), Group A and Group B streptococcus (including *Streptococcus pneumoniae, Streptococcus agalactiae* and *Streptococcus pyogenes* as described, for example, in Watson *et al.* (2000) *Pediatr. Infect. Dis. J.* 19:331-332; Rubin *et al.* (2000) *Pediatr Clin. North Am.* 47:269-284; Jedrzejas *et al.* (2001) *Microbiol Mol Biol Rev* 65:187-207; Schuchat (1999) *Lancet* 353:51-56; GB patent applications 0026333.5; 0028727.6; 015640.7; Dale *et al.* (1999) *Infect Dis Clin North Am* 13:227-1243; Ferretti *et al.* (2001) *PNAS USA* 98:4658-4663), pertussis (*See, e.g.*, Gusttafsson *et al.* (1996) *N. Engl. J. Med.* 334:349-355; Rappuoli *et al.* (1991) *TIBTECH* 9:232-238), meningitis, *Moraxella catarrhalis* (*See, e.g.*, McMichael (2000) *Vaccine* 19 Suppl. 1:S101-107) and other pathogenic states,

including, without limitation, Neisseria meningitides (A, B, C, Y), *Neisseria gonorrhoeae* (*See, e.g.*, WO 99/24578; WO 99/36544; and WO 99/57280), Helicobacter pylori (*e.g.*, CagA, VacA, NAP, HopX, HopY and/or urease as described, for example, WO 93/18150; WO 99/53310; WO 98/04702) and *Haemophilus influenza*. Hemophilus influenza type B (HIB) (See, *e.g.*, Costantino *et al.* (1999) *Vaccine* 17:1251-1263), *Porphyromonas gingivalis* (Ross *et al.* (2001) *Vaccine* 19:4135-4132) and combinations thereof.

Non-limiting examples of viral pathogens which may be used in the invention include meningitis, rhinovirus, influenza (Kawaoka *et al.*, *Virology* (1990) 179:759-767; Webster *et al.*, "Antigenic variation among type A influenza viruses," p. 127-168. In: P. Palese and D.W. Kingsbury (ed.), Genetics of influenza viruses. Springer-Verlag, New York), respiratory syncytial virus (RSV), parainfluenza virus (PIV), rotavirus (*e.g.*, VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 or NSP5 and other rotavirus antigens, for example as described in WO 00/26380) and the like. Antigens derived from other viruses will also find use in the present invention, such as without limitation, proteins from members of the families Picomaviridae (*e.g.*, polioviruses, *etc.* as described, for example, in Sutter *et al.* (2000) *Pediatr Clin North Am* 47:287-308; Zimmerman & Spann (1999) *Am Fam Physician* 59:113-118; 125-126); Caliciviridae; Togaviridae (*e.g.*, rubella virus, *etc.*); Flaviviridae, including the genera flavivirus (*e.g.*, yellow fever virus, Japanese encephalitis virus, serotypes of Dengue virus, tick borne encephalitis virus, West Nile virus, St. Louis encephalitis virus); pestivirus (*e.g.*, classical porcine fever virus, bovine viral diarrhea virus, border disease virus); and hepacivirus (*e.g.*, hepatitis A, B and C as described, for example, in US Patent Nos. 4,702,909; 5,011,915; 5,698,390; 6,027,729; and 6,297,048); Parvovirus (*e.g.*, parvovirus B19); Coronaviridae; Reoviridae; Bimaviridae; Rhabodoviridae (*e.g.*, rabies virus, *etc.* as described for example in Dressen *et al.* (1997) *Vaccine* 15 Suppl:s2-6; MMWR Morb Mortal Wkly Rep. 1998 Jan 16:47(1):12, 19); Filoviridae; Paramyxoviridae (*e.g.*, mumps virus, measles virus, respiratory syncytial virus, *etc.* as described in Chapters 9 to 11 of *Vaccines*, 1998, eds. Plotkin & Mortimer (ISBN 0-7216-1946-0); Orthomyxoviridae (*e.g.*, influenza virus types A, B and C, *etc.* as described in Chapter 19 of *Vaccines*, 1998, eds. Plotkin & Mortimer (ISBN 0-7216-1946-0),.); Bunyaviridae; Arenaviridae; Retroviradae (*e.g.*, HTLV-1; HTLV-11; HIV-1 (also known as HTLV-III, LAV, ARV, HTI,R, *etc.*)), including but not limited to antigens from the isolates HIVIIIb, HIVSF2, HIVLAV, HIVI-AL, I-IIVMN, SF162); HIV- I CM235, HIV- I US4; HIV-2; simian immunodeficiency virus (SIV) among others. Additionally, antigens may also be derived from human papilloma virus (HPV) and the tick-borne encephalitis viruses. See, *e.g.* Virology, 3rd Edition (W.K. Joklik ed. 1988); Fundamental Virology, 2nd Edition (B.N. Fields and D.M. Knipe, eds, 1991), for a description of these and other viruses.

Proteins may also be derived from the herpesvirus family, including proteins derived from herpes simplex virus (HSV) types 1 and 2, such as HSV-1 and HSV-2 glycoproteins gB, gD and gH; antigens derived from varicella zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) including CMV gB and gH (See, US Patent No. 4,689,225 and PCT Publication WO 89/07143); and antigens derived from other human herpesviruses such as HHV6 and HHV7. (See, *e.g.* Chee *et al.*, *Cytomegaloviruses* (J.K. McDougall, ed., Springer-Verlag 1990) pp. 125-169, for a review of the protein coding content of cytomegalovirus; McGeoch *et al.*, *J. Gen. Virol.* (1988) 69:1531-1574, for a discussion of the various HSV-1 encoded proteins; US Patent No. 5,171,568 for a discussion of HSV-1 and HSV-2 gB and gD proteins and the genes encoding therefor; Baer *et al.*, *Nature* (1984) 310:207-211, for the identification of protein coding sequences in an EBV genome; and Davison and Scott, *J. Gen. Virol.* (1986) 67:1759-1816, for a review of VZV). Herpes simplex virus (HSV) rgD2 is a recombinant protein produced in genetically engineered Chinese hamster ovary cells. This protein has the normal anchor region truncated, resulting in a glycosylated protein secreted into tissue culture medium. The gD2 can be purified in the CHO medium to greater than 90% purity. Human immunodeficiency virus (HIV) env-2-3 is a recombinant form of the HIV enveloped protein produced in genetically engineered *Saccharomyces cerevisae*. This protein represents the entire protein region of HIV gp120 but is non-glycosylated and denatured as purified from the yeast. HIV gp120 is a fully glycosylated, secreted form of gp120 produced in CHO cells in a fashion similar to the gD2 above. Additional HSV antigens suitable for use in immunogenic compositions are described in PCT Publications W0 85/04587 and W0 88/02634, the disclosures of which are incorporated herein by reference in their entirety. Mixtures of gB and gD antigens, which are truncated surface antigens lacking the anchor regions, are particularly preferred.

Antigens from the hepatitis family of viruses, including hepatitis A virus (HAV) (*See, e.g.*, Bell *et al.* (2000) *Pediatr Infect Dis. J.* 19:1187-1188; Iwarson (1995) *APMIS* 103:321-326), hepatitis B virus (HBV) (*See, e.g.*, Gerlich *et al.* (1990) *Vaccine* 8 Suppl:S63-68 & 79-80), hepatitis C virus (HCV) (*See, e.g.*, PCT/US88/04125, published European application number 318216), the delta hepatitis virus (HDV), hepatitis E virus (HEV) and hepatitis G virus (HGV), can also be conveniently used in the techniques described herein. By way of example, the viral genomic sequence of HCV is known, as are methods for obtaining the sequence. See, *e.g.*, International Publication Nos. WO 89/04669; WO 90/11089; and WO 90/14436. Also included in the invention are molecular variants of such polypeptides, for example as described in PCT/US99/31245; PCT/US99/31273 and PCT/US99/31272. The HCV genome encodes several viral proteins, including E1 (also known as E) and E2 (also known as E2/NSI) and an N-terminal nucleocapsid protein (termed "core") (see, Houghton *et al.*, *Hepatology* (1991) 14:381-388, for a discussion of HCV proteins, including E1 and E2). Similarly, the sequence for the δ-antigen

from HDV is known (see, *e.g.*, US Patent No. 5,378,814) and this antigen can also be conveniently used in the present composition and methods. Additionally, antigens derived from HBV, such as the core antigen, the surface antigen, SAg, as well as the presurface sequences, pre-S1 and pre-S2 (formerly called pre-S), as well as combinations of the above, such as SAg/pre-S1, SAg/pre-S2, SAg/pre-S1/pre-S2, and pre-S1/pre-S2, will find use herein. See, *e.g.*, "HBV Vaccines - from the laboratory to license: a case study" in Mackett, M. and Williamson, J.D., *Human Vaccines and Vaccination*, pp. 159-176, for a discussion of HBV structure; and US Patent Nos. 4,722,840, 5,098,704, 5,324,513, incorporated herein by reference in their entireties; Beames *et al.*, *J. Virol.* (1995) 69:6833-6838, Birnbaum *et al.*, *J. Virol.* (1990) 64:3319-3330; and Zhou *et al.*, *J. Virol.* (1991) 65:5457-5464. Each of these proteins, as well as antigenic fragments thereof, will find use in the present composition and methods.

Influenza virus is another example of a virus for which the present invention will be particularly useful. Specifically, the envelope glycoproteins HA and NA of influenza A are of particular interest for generating an immune response. Numerous HA subtypes of influenza A have been identified (Kawaoka *et al.*, *Virology* (1990) 179:759-767; Webster *et al.*, "Antigenic variation among type A influenza viruses," p. 127-168. In: P. Palese and D.W. Kingsbury (ed.), *Genetics of influenza viruses*. Springer-Verlag, New York). Thus, proteins derived from any of these isolates can also be used in the compositions and methods described herein.

Non-limiting examples of parasitic antigens include those derived from organisms causing malaria and Lyme disease.

The methods of the invention comprise administering an immunogenic composition comprising a SARS viral antigen (including one or more of an inactivated SARS virus, an attenuated SARS virus, a split SARS virus preparation or a recombinant or purified subunit formulation of one or more SARS viral antigens) to an animal. The immunogenic compositions used in the invention can comprise an immunologically effective amount of the SARS viral antigen. An "immunologically effective amount" is an amount sufficient to allow the mammal to raise an immune response to the SARS antigen.

The immune response preferably involves the production of antibodies specific to the SARS antigen. The amount of antibodies produced will vary depending on several factors including the animal used, the presence of an adjuvant, *etc.*

The immunogenic compositions of the invention may further comprise one or more adjuvants.

The immunogenic compositions of the invention may be administered mucosally. Suitable routes of mucosal administration include oral, intranasal, intragastric, pulmonary, intestinal, rectal, ocular and vaginal routes. The immunogenic composition may be adapted for mucosal administration. For instance, where the composition is for oral administration, it may be in the

form of tablets or capsules, optionally enteric-coated, liquid, transgenic plants, *etc.* Where the composition is for intranasal administration, it may be in the form of a nasal spray, nasal drops, gel or powder.

The immunogenic compositions of the invention may be administered parenterally. Suitable routes of parenteral administration include intramuscular (IM), subcutaneous, intravenous, intraperitoneal, intradermal, transcutaneous, and transdermal (*see e.g.*, International patent application WO 98/20734) routes, as well as delivery to the interstitial space of a tissue. The immunogenic composition may be adapted for parenteral administration, for instance in the form of an injectable that may be sterile and pyrogen free.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphoshpates, orthophosphates), sulphates, *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine design:the subunit and adjuvant approach* (1995) Powell & Newman. ISBN 0-306-44867-X.), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See WO00/23105.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Frey *et al.*, "Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", *Vaccine* (2003) 21:4234-4237.

Particularly preferred adjuvants for use in the compositions are submicron oil-inwater emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v Tween 80 ™ (polyoxyelthylenesorbitan monooleate), and/or 0.25-1.0% Span 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-huydroxyphosphophoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water

emulsion known as "MF59" (International Publication No. WO 90/14837; US Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott *et al.*, "MF59 -- Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (*e.g.*, 4.3%), 0.25-0.5% w/v Tween 80™, and 0.5% w/v Span 85™ and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 μg/dose, more preferably 0-250 μg/dose and most preferably, 0-100 μg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 μg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v Tween 80™, and 0.75% w/v Span 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 μg MTP-PE per dose.

Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in International Publication No. WO 90114837 and US Patent Nos. 6,299,884 and 6,45 1,325, incorporated herein by reference in their entireties.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7,

QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in US Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See WO00/07621.

A review of the development of saponin based adjuvants can be found at Barr, *et al.*, "ISCOMs and other saponin based adjuvants", Advanced Drug Delivery Reviews (1998) <u>32</u>:247-271. See also Sjolander, *et al.*, "Uptake and adjuvant activity of orally delivered saponin and ISCOM vaccines", Advanced Drug Delivery Reviews (1998) <u>32</u>:321-338.

<u>D. Bacterial or Microbial Derivatives</u>

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.

(2) Lipid A Derivatives

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Meraldi *et al.*, "OM-174, a New Adjuvant with a Potential for Human Use, Induces a Protective Response with Administered with the Synthetic C-Terminal Fragment 242-310 from the circumsporozoite protein of Plasmodium berghei", Vaccine (2003) <u>21</u>:2485-2491; and Pajak, *et al.*, "The Adjuvant OM-174 induces both the migration and maturation of murine dendritic cells in vivo", Vaccine (2003) <u>21</u>:836-842.

(3) Immunostimulatory oligonucleotides

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or

oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See Kandimalla, *et al.*, "Divergent synthetic nucleotide motif recognition pattern: design and development of potent immunomodulatory oligodeoxyribonucleotide agents with distinct cytokine induction profiles", Nucleic Acids Research (2003) 31(9): 2393-2400; WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg, "CpG motifs: the active ingredient in bacterial extracts?", Nature Medicine (2003) 9(7): 831-835; McCluskie, *et al.*, "Parenteral and mucosal prime-boost immunization strategies in mice with hepatitis B surface antigen and CpG DNA", FEMS Immunology and Medical Microbiology (2002) 32:179-185; WO 98/40100; US Patent No. 6,207,646; US Patent No. 6,239,116 and US Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kandimalla, *et al.*, "Toll-like receptor 9: modulation of recognition and cytokine induction by novel synthetic CpG DNAs", Biochemical Society Transactions (2003) 31 (part 3): 654-658. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, *et al.*, "CpG-A-Induced Monocyte IFN-gamma-Inducible Protein-10 Production is Regulated by Plasmacytoid Dendritic Cell Derived IFN-alpha", J. Immunol. (2003) 170(8):4061-4068; Krieg, "From A to Z on CpG", TRENDS in Immunology (2002) 23(2): 64-65 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, Kandimalla, *et al.*, "Secondary structures in CpG oligonucleotides affect immunostimulatory activity", BBRC (2003) 306:948-953; Kandimalla, *et al.*, "Toll-like receptor 9: modulation of recognition and cytokine induction by novel synthetic GpG DNAs", Biochemical Society Transactions (2003) 31(part 3):664-658; Bhagat *et al.*, "CpG penta- and hexadeoxyribonucleotides as potent immunomodulatory agents" BBRC (2003) 300:853-861 and WO 03/035836.

(4) ADP-ribosylating toxins and detoxified derivatives thereof.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (*i.e.*, E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO

98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references, each of which is specifically incorporated by reference herein in their entirety: Beignon, *et al.*, "The LTR72 Mutant of Heat-Labile Enterotoxin of Escherichia coli Enahnces the Ability of Peptide Antigens to Elicit CD4+ T Cells and Secrete Gamma Interferon after Coapplication onto Bare Skin", Infection and Immunity (2002) 70(6):3012-3019; Pizza, *et al.*, "Mucosal vaccines: non-toxic derivatives of LT and CT as mucosal adjuvants", Vaccine (2001) 19:2534-2541; Pizza, *et al.*, "LTK63 and LTR72, two mucosal adjuvants ready for clinical trials" Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten *et al.*, "Transcutaneous Immunization with Bacterial ADP-Ribosylating Exotoxins, Subunits and Unrelated Adjuvants", Infection and Immunity (2000) 68(9):5306-5313; Ryan *et al.*, "Mutants of Escherichia coli Heat-Labile Toxin Act as Effective Mucosal Adjuvants for Nasal Delivery of an Acellular Pertussis Vaccine: Differential Effects of the Nontoxic AB Complex and Enzyme Activity on Th1 and Th2 Cells" Infection and Immunity (1999) 67(12):6270-6280; Partidos *et al.*, "Heat-labile enterotoxin of Escherichia coli and its site-directed mutant LTK63 enhance the proliferative and cytotoxic T-cell responses to intranasally co-immunized synthetic peptides", Immunol. Lett. (1999) 67(3):209-216; Peppoloni *et al.*, "Mutants of the Escherichia coli heat-labile enterotoxin as safe and strong adjuvants for intranasal delivery of vaccines", Vaccines (2003) 2(2):285-293; and Pine *et al.*, (2002) "Intranasal immunization with influenza vaccine and a detoxified mutant of heat labile enterotoxin from Escherichia coli (LTK63)" J. Control Release (2002) 85(1-3):263-270. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini *et al.*, Mol. Microbiol (1995) 15(6):1165-1167, specifically incorporated herein by reference in its entirety.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) *J. Cont. Rele.* 70:267-276) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. *E.g.*, WO99/27960.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in US Patent No. 6,090,406, US Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. WO99/52549. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Andrianov *et al.*, "Preparation of hydrogel microspheres by coacervation of aqueous polyphophazene solutions", Biomaterials (1998) 19(1-3):109-115 and Payne *et al.*, "Protein Release from Polyphosphazene Matrices", Adv. Drug. Delivery Review (1998) 31(3):185-196.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues, described further in Stanley, "Imiquimod and the

imidazoquinolones: mechanism of action and therapeutic potential" Clin Exp Dermatol (2002) 27(7):571-577 and Jones, "Resiquimod 3M", Curr Opin Investig Drugs (2003) 4(2):214-218.

M. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Niikura *et al.*, "Chimeric Recombinant Hepatitis E Virus-Like Particles as an Oral Vaccine Vehicle Presenting Foreign Epitopes", Virology (2002) 293:273-280; Lenz *et al.*, "Papillomarivurs-Like Particles Induce Acute Activation of Dendritic Cells", Journal of Immunology (2001) 5246-5355; Pinto, *et al.*, "Cellular Immune Responses to Human Papillomavirus (HPV)-16 L1 Healthy Volunteers Immunized with Recombinant HPV-16 L1 Virus-Like Particles", *Journal of Infectious Diseases* (2003) 188:327-338; and Gerber *et al.*, "Human Papillomavrisu Virus-Like Particles Are Efficient Oral Immunogens when Coadministered with Escherichia coli Heat-Labile Entertoxin Mutant R192G or CpG", Journal of Virology (2001) 75(10):4752-4760. Virosomes are discussed further in, for example, Gluck *et al.*, "New Technology Platforms in the Development of Vaccines for the Future", Vaccine (2002) 20:B10 –B16.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (WO99/11241);

(2) a saponin (*e.g.*., QS21) + a non-toxic LPS derivative (*e.g.*, 3dMPL) (see WO 94/00153);

(3) a saponin (*e.g.*., QS21) + a non-toxic LPS derivative (*e.g.*, 3dMPL) + a cholesterol;

(4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) (WO98/57659);

(5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);

(6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

As mentioned above, adjuvants suitable for use in the invention may also include one or more of the following:

- *E.coli* heat-labile enterotoxin ("LT"), or detoxified mutants thereof, such as the K63 or R72 mutants;

- cholera toxin ("CT"), or detoxified mutants thereof;

- microparticles (i.e., a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (e.g. a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone *etc.*);

- a polyoxyethylene ether or a polyoxyethylene ester (see International patent application WO 99/52549);

- a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (see International patent application WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (see International patent application WO 01/21152);

- chitosan (e.g. International patent application WO 99/27960)

- an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) and a saponin (see International patent application WO 00/62800)

- immunostimulatory double stranded RNA.

- aluminum compounds (e.g. aluminum hydroxide, aluminum phosphate, aluminum hydroxyphosphate, oxyhydroxide, orthophosphate, sulfate *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine design: the subunit and adjuvant aproach,* eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X) (hereinafter "Vaccine design"), or mixtures of different aluminum compounds, with the compounds taking any suitable form (e.g. gel, crystalline, amorphous *etc.*), and with adsorption being preferred;

- MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer) (see Chapter 10 of Vaccine design; see also International patent application WO 90/14837);

- liposomes (see Chapters 13 and 14 of *Vaccine design*);

- ISCOMs (see Chapter 23 of *Vaccine design*);

- SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion (see Chapter 12 of *Vaccine design*);

- Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™);

- saponin adjuvants, such as QuilA or QS21 (see Chapter 22 of *Vaccine design*), also known as Stimulon™;

- ISCOMs, which may be devoid of additional detergent (WO 00/07621);

- complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA);

- cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. interferon-γ), macrophage colony stimulating factor, tumor necrosis factor, etc. (see Chapters 27 & 28 of *Vaccine design*);

- monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) (e.g. chapter 21 of *Vaccine design*);

- combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (European patent applications 0835318, 0735898 and 0761231);

- oligonucleotides comprising CpG motifs (see Krieg (2000) Vaccine, 19:618-622; Krieg (2001) Curr. Opin. Mol. Ther., 2001, 3:15-24; WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581, etc.) *i.e.* containing at least one CG dinucleotide,

- a polyoxyethylene ether or a polyoxyethylene ester (International patent application WO99/52549);

- a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (International patent application WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO 01/21152);

- an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) and a saponin (WO00/62800);

- an immunostimulant and a particle of metal salt (International patent application WO00/23105);

- a saponin and an oil-in-water emulsion (WO 99/11241);

- a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) (WO 98/57659).

Other adjuvants suitable for mucosal or parenteral administration are also available (*e.g.* see chapter 7 of *Vaccine design: the subunit and adjuvant aproach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X).

Mutants of LT are preferred mucosal adjuvants, in particular the "K63" and "R72" mutants (*e.g.* see International patent application WO 98/18928), as these result in an enhanced immune response.

Microparticles are also preferred mucosal adjuvants. These are preferably derived from a poly(α-hydroxy acid), in particular, from a poly(lactide) ("PLA"), a copolymer of D,L-lactide and glycolide or glycolic acid, such as a poly(D,L-lactide-co-glycolide) ("PLG" or "PLGA"), or a copolymer of D,L-lactide and caprolactone. The microparticles may be derived from any of various polymeric starting materials which have a variety of molecular weights and, in the case of the copolymers such as PLG, a variety of lactide:glycolide ratios, the selection of which will be largely a matter of choice, depending in part on the coadministered antigen.

The SARS virus (inactivated or attenuated), viral antigens, antibodies or adjuvants of the invention may be entrapped within the microparticles, or may be adsorbed to them. Entrapment within PLG microparticles is preferred. PLG microparticles are discussed in further detail in Morris *et al.*, (1994), *Vaccine*, 12:5-11, in chapter 13 of *Mucosal Vaccines*, eds. Kiyono *et al.*, Academic Press 1996 (ISBN 012410587), and in chapters 16 & 18 of *Vaccine design: the subunit and adjuvant aproach*, eds. Powell & Newman, Plenum Press 1995 (ISBN 0-306-44867-X).

LT mutants may advantageously be used in combination with microparticle-entrapped antigen, resulting in significantly enhanced immune responses.

Aluminium compounds and MF59 are preferred adjuvants for parenteral use.

The composition may include an antibiotic.

The immunogenic compositions of the invention may be administered in a single dose, or as part of an administration regime. The regime may include priming and boosting doses, which may be administered mucosally, parenterally, or various combinations thereof.

The methods of the invention further comprise treating or preventing a SARS virus-related disease by administering to an animal a composition comprising an effective amount of the antibodies of the invention. An "effective amount" of the antibodies of the invention is an amount sufficient to provide passive immunization protection or treatment to the animal. Preferably, the antibodies of the invention are specific to the SARS viral antigen.

Methods of treatment may combine both immunogenic compositions and antibody compositions. Accordingly the invention comprises a method for treating or preventing a SARS virus-related disease comprising administering an immunogenic composition comprising an immunologically effective amount of a SARS viral antigen and administering an effective amount of antibodies specific to SARS viral antigen. The immunogenic composition and the antibodies may be administered together or separately. The invention further comprises a composition comprising an immunogenic composition comprising an immunologically effective amount of a SARS viral antigen and further comprising an effective amount of antibodies specific to a SARS viral antigen.

The SARS viral antigens and antibodies of the invention may also be administered in polynucleotide form. The SARS viral antigens and/or antibody proteins are then expressed *in vivo*.

The SARS viral antigens and the antibodies of the invention can also be delivered using one or more gene vectors, administered via nucleic acid immunization or the like using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, US Patent Nos. 5,399,346, 5,580,859, 5,589,466. The constructs can be delivered (*e.g.*, injected) either subcutaneously, epidermally, intradermally, intramuscularly, intravenous, mucosally (such as nasally, rectally and vaginally), intraperitoneally, orally or combinations thereof. Intramuscular injection of 25μg plasmid DNA encoding spike antigens, in 200μl PBS pH 7.4, at weeks 0, 3 and 6, has been described for mice by Yang *et al.* (2004) *Nature* 428:561-564.

An exemplary replication-deficient gene delivery vehicle that may be used in the practice of the present invention is any of the alphavirus vectors, described in, for example, US Patent Nos. 6,342,372; 6,329,201 and International Publication WO 01/92552.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described (US Patent No. 5,219,740; Miller & Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa *et al.*, *Virology* (1991) 180:849-852; Burns *et al.*, *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie & Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett *et al.*, *J. Virol.* (1993) 67:5911-5921; Mittereder *et al.*, *Human Gene Therapy* (1994)

5:717-729; Seth *et al.*, *J. Virol.* (1994) 68:933-940; Barr *et al.*, *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich *et al.*, *Human Gene Therapy* (1993) 4:461-476). Adenoviral delivery of codon-optimsed versions of the genes encoding SARS coronavirus structural antigens spike S1, membrane protein and nucleocapsid protein has been investigated in rhesus macaques and found to invoke a strong neutralizing antibody response (Gao *et al.* (2003) *Lancet* 362(9399):1895-1896).

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV vectors can be readily constructed using techniques well known in the art. See, *e.g.*, US Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski *et al.*, *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent *et al.*, *Vaccines 90* (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou *et al.*, *J. Exp. Med.* (1994) 179:1867-1875.

Another vector system useful for delivering polynucleotides, mucosally and otherwise, is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P.A., *et al.* (US Patent No. 5,676,950, issued October 14, 1997, herein incorporated by reference) as well as the vaccinia virus and avian poxviruses. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the SARS antigen or antibody or antibody coding sequence is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells that are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK^- recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver genes encoding the SARS viral antigens or antibodies of the invention. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia

viruses. See, *e.g.*, WO 91/12882; WO 89/03429; and WO 92/03545. Picornavirus-derived vectors can also be used. (See, *e.g.*, US Patent Nos. 5,614,413 and 6,063,384).

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael *et al.*, *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest (for example, a SARS viral antigen or antibody expression cassette) in a host cell. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA that is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See, *e.g.*, Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst *et al.*, *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase that in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, *e.g.*, International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao *et al.*, *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen *et al.*, *Nuc. Acids Res.* (1994) 22:2114-2120; and US Patent No. 5,135,855.

The immunogenic compositions of the invention may further comprise diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or

emulsifying agents, pH buffering substances, and the like may be included in the immunogenic composition.

The immunogenic compositions used in the invention can be administered to an animal. Animals suitable for use in the methods of the invention include humans and other primates, including non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses, domestic animals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese and the like. Animals suitable for use in the invention can be of any age, including both adult and newborn. Transgenic animals can also be used in the invention.

The immunogenic compositions of the invention can be used to treat or prevent SARS virus-related diseases.

The compositions of the invention are preferably pharmaceutically acceptable and pharmacologically acceptable. In particularly, the compositions are preferably not biologically or otherwise undesirable, *i.e.*, the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art. Compositions of the invention can also contain liquids or excipients, such as water, saline, glycerol, dextrose, ethanol, or the like, singly or in combination, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes can also be used as a carrier for a composition of the invention.

SARS specific reagents and analytical assays may be used in the manufacture and testing of the vaccines of the invention. Such analytical assays include, for example: 1) virus titration and plaque assays for quantitation of infectious virus particles, 2) a neutralization assay with constant virus and varying serum dilutions, 3) a two step RT-PCR system (Light Cycler-Roche) for detection of negative strand viral RNA, with the target sequence located within the N gene, providing highest possible sensitivity, and 4) ELISA and western blot assays for detection and qualification of viral proteins.

In addition, rabbit polyclonal antiserum has been generated to obtain antibody reagents (and demonstrate induction of neutralizing antibodies) against the SARS-CoV. A sample protocol for generating such reagents is set forth below. The virus is first cultivated in suitable

cell culture, such as Vero cells, and pelleted through a 20% sucrose (w/v) cushion. The pellet is then subjected to a glycerol potassium-tartrate gradient for further purification. The virus-containing fraction is then diluted and pelleted by ultracentrifugation. The pellet is then dissolved in PBS and the virus is inactivated with $C_3H_4O_2$ (beta-propiolactone, BPL). Two rabbits are immunized subcutaneously (SC) on day 0, 14, and 28 with 1×10^9 inactivated viral particles mixed with IFA as adjuvant. Rabbits are bled on days 0 (pre-inoculation), 13, 28, and 35 (1 week after 3rd immunization). Sera obtained from this protocol were tested for their reactivity against SARS-CoV proteins in western blots and found to react with the major structural proteins spike (S), membrane (M), and nucleocapsid (N).

J. Emerging coronavirus vaccines

The SARS epidemic has lead to increased awareness of viral infections caused by coronaviruses. The vaccines of the invention may be adapted to prevent or treat emerging strains of coronavirus, including emerging strains of SARS virus.

The invention provides a vaccine comprising an inactivated (or killed) human coronavirus, an attenuated human coronavirus, a split human coronavirus preparation, or a recombinant or purified subunit formulation of one or more antigens from a human coronavirus, wherein the human coronavirus is not the SARS coronavirus. Optionally, the human coronavirus is not the 229E coronavirus. Optionally, the human coronavirus is not the OC43 coronavirus. Optionally, the human coronavirus is not the NL63 coronavirus. Thus the invention provides a vaccine as defined above, wherein the human coronavirus is not the SARS coronavirus, is not the 229E coronavirus, is not the OC43 coronavirus and is not the NL63 coronavirus. Such vaccines are useful for preventing and/or treating emerging human coronavirus infections.

The invention also provides a vaccine comprising: (a) an inactivated (or killed) human coronavirus, an attenuated human coronavirus, a split human coronavirus preparation, or a recombinant or purified subunit formulation of one or more antigens from a human coronavirus, wherein the human coronavirus is not the SARS coronavirus, as defined above; and (b) an inactivated (or killed) human coronavirus, an attenuated human coronavirus, a split human coronavirus preparation, or a recombinant or purified subunit formulation of one or more antigens from a human coronavirus, wherein the human coronavirus is the SARS coronavirus. Such vaccines are useful for preventing and/or treating both SARS and other human coronaviruses.

As well as providing vaccines comprising antigens from more than one type of coronavirus, the invention also provides vaccines comprising antigens from more than one strain of the same coronavirus *e.g.* different strains of the SARS coronavirus, or different strains of a coronavirus other than the SARS coronavirus. In one embodiment, the vaccine comprises antigens from at least two strains of coronavirus, or at least three strains of coronavirus. In one

embodiment, the vaccine comprises antigens from at least two types of coronavirus. In one embodiment, the vaccine comprises at least one antigen from each of the known types of coronaviruses (type I, type II and type III). Such vaccines follow the model of current influenza vaccines.

The selection of coronaviruses and/or coronavirus strains for use in vaccines of the invention can be based on various criteria. For instance, selection may be based on viruses and/or strains that have been detected in the geographical region (*e.g.* northern or southern hemisphere, a particular country, *etc.*) where the vaccine targeted. Selection may be based on the results of animal surveillance *e.g.* of viruses detected in cat populations. Selection may be based on the results of clinical surveillance *e.g.* of viruses detected in patients hospitalized with respiratory infection. Selection may be performed every year *e.g.* prior to winter. Vaccines may also be administered yearly, again following the model of current influenza vaccines.

Preferred vaccines are sufficiently immunogenic to provide a neutralizing immune response, and more preferably a protective and/or therapeutic immune response. Particularly preferred vaccines meet the efficacy requirements that may be specified by the WHO from time to time.

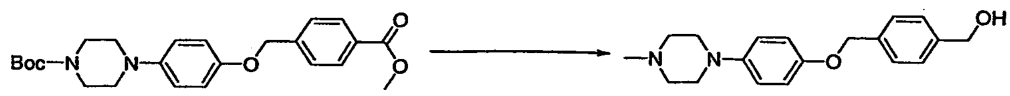
A preferred subunit antigen for inclusion in vaccines of the invention is a purified spike protein, more preferably in oligomeric (*e.g.* trimeric) form. The spike protein may or my not be cleaved *e.g.* into its S1 and S2 products.

The techniques disclosed above for selecting viruses and/or strains for production of vaccines can also be used to select appropriate viruses and/or strains from which HR1 and HR2 sequences can be obtained for providing therapeutic peptides, as disclosed above.

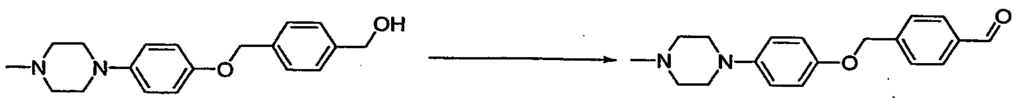
III. DIAGNOSTIC COMPOSITIONS AND METHODS OF THE INVENTION

The invention provides methods for detecting the SARS coronavirus. Detection in patient samples can be used to detect and diagnose infections by the virus. Detection in donated blood can be used to prevent inadvertent transmission of the virus during blood transplant procedures Detection methods fall into three main categories: detection of SARS virus nucleic acids; detection of SARS virus proteins; and detection of anti-SARS virus immune responses. The invention provides all such methods.

As used herein when referring to nucleotide sequences, particularly oligonucleotide probes and primers, "similar" sequences includes those sequences that are at least 90% identical to known SARSV genomic sequence and includes sequences that are at least 95 % identical, at least 99% identical and 100% identical to the SARSV genomic sequence over the length of the probe or primer.



To a slurry of $LiAlH_4$ (4 equivalents) in dry THF, cooled to 0 °C under N_2, was slowly added drop-wise a solution of methyl 4-[4-(1-BOC-piperazin-4-yl)phenoxymethyl]benzoate (1 equivalent) in dry THF. Once the addition was complete, the slurry was heated to reflux at 80 °C for 1 hour. The slurry was subsequently cooled to 0 °C and treated with water, 10% aq. NaOH and with water again. The resulting solids were filtered, and the filtrate was diluted with chloroform, washed with brine, dried over $MgSO_4$ and concentrated, providing the crude 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzyl alcohol that was used without purification.

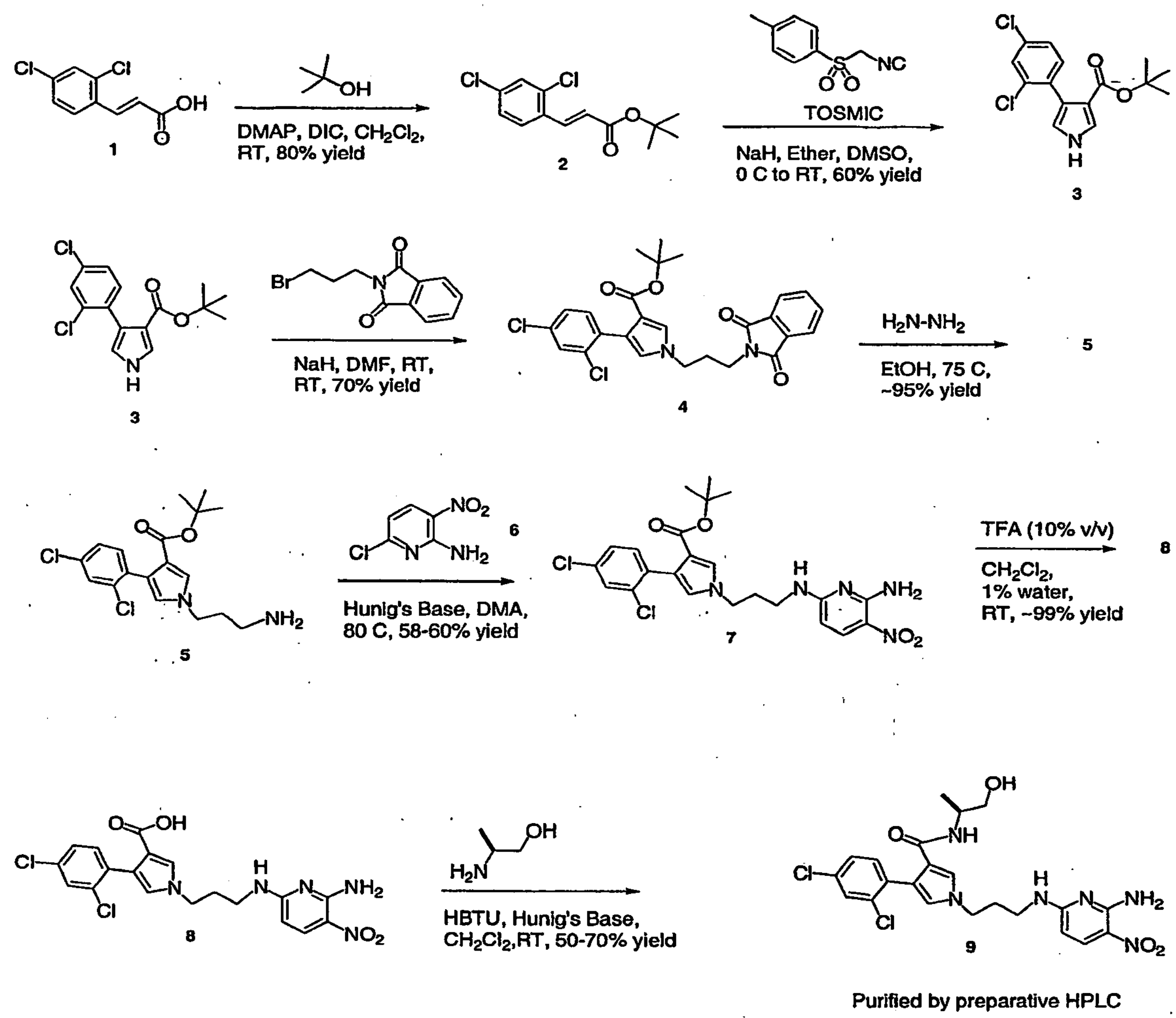


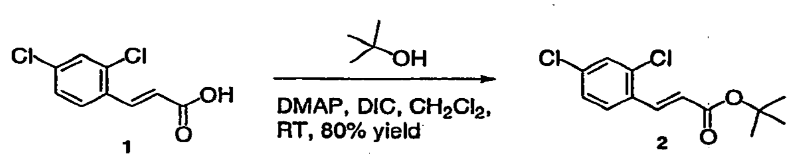
To a solution of DMSO (2.6 equivalents) in dry DCM , cooled to –78 °C under N_2 was added oxalyl chloride (1.1 equivalents) in DCM drop-wise. The solution was stirred at –78 °C for 5 minutes before a solution of 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzyl alcohol (1 equivalent) in DCM was added drop-wise, and allowed to stir at –78 °C for another 30 minutes. Triethylamine (2.5 equivalents) was slowly dripped in before allowing the solution to reach ambient temperatures. The solution was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated to provide the crude 4-[4-(4-methylpiperazin-1-yl)phenoxymethyl]benzaldehyde that was converted to thiosemicarbazones according to Scheme 7.

PYRROLES

Scheme 15

Synthesis of Pyrrole

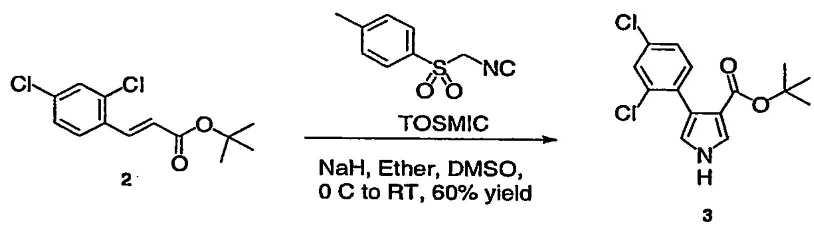




Preparation of *tert*-butyl (2E)-3-(2,4-dichlorophenyl)prop-2-enoate (2).

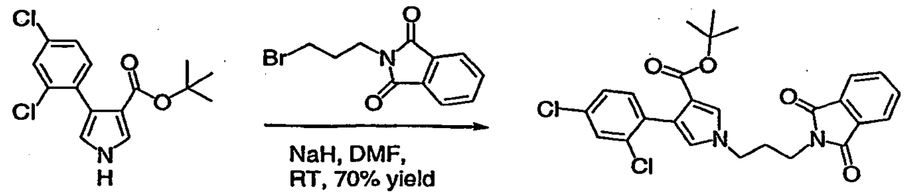
Neat DIC (1.4 eq) was added to a well stirred solution of cinnamate (1 eq), t-butyl alcohol (4 eq), DMAP (1.4 eq) and CH_2Cl_2 under argon at rt. (Note - The cinnamate must be completely in solution that may require gentle warming. Allow the solution to cool to room temperature before adding the DIC. To avoid an exotherm on larger scales, it may be prudent to

dilute the DIC with CH_2Cl_2 before the addition and have an ice bath ready.) After stirring for 8 hours, the reaction develops a white precipitate. The reaction may be monitored by TLC eluting with 25% EtOAc/Hexane (R_f of product was 0.9). The entire reaction was loaded into a separatory funnel (washing with CH_2Cl_2). The organic mixture was washed with citrate, sat. aq. $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness to give the crude product as an oil. The crude oil was mixed with hexane and stirred for 30 min. The precipitate that forms was filtered over celite and the filtrate was evaporated. The hexane mixture was loaded onto a filter plug of silica and eluted with EtOAc/hexane (97:2 v/v). The first eluted UV active fractions are collected and evaporated to give >99% pure **2** (75-80% yields).



Preparation of *tert*-butyl 4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (3).

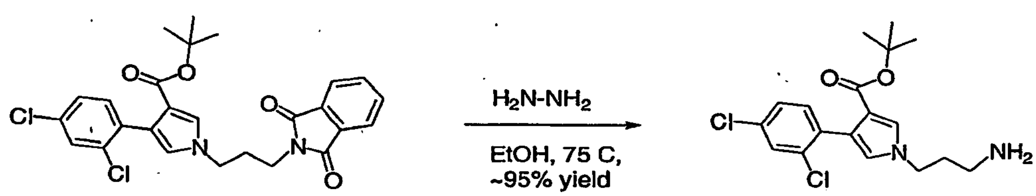
Dry ether was added to NaH (1.5 eq as the oil dispersion) under argon. After decanting off the ether via syringe, the NaH was suspended again with fresh ether under argon. A solution of TOSMIC (1.1 eq) and 2 (1 eq) dissolved in a mixture of ether and DMSO was added dropwise to the stirred suspension of NaH at 0 °C over 20-30 min. The addition was mildly exothermic and evolved gas. After the addition, the reaction was allowed to warm to ambient rt. The progress of the reaction was followed by TLC (25% EtOAc/Hexane, the UV active product was at $R_f = 0.4$) and LCMS until done (~2-3 h). Upon completion, the reaction was carefully quenched with sat. aq. NH_4Cl (added slowly to avoid strong gas evolution and exotherm) and diluted with ether. The layers were separated and the organic phase was washed with sat. aq. $NaHCO_3$, water, and brine. The crude dark solid can be purified by recrystallization. Best results were achieved either through recrystallization directly from a mixture of hot EtOAc/hexane (1:3 v/v) or by dissolving the crude product in minimal hot EtOAc followed by addition of hexane (~2 volumes of hexane based on the volume of EtOAc). The hot solutions were allowed to cool to room temperature and age over night. The crystals were first filtered and then washed with hexane giving 99% pure product in 60-70 % yield.



3 4

Preparation of *tert*-butyl 4-(2,4-dichlorophenyl)-1-[3-(1,3-dioxobenzo[c]azolin-2-yl)propyl]pyrrole-3-carboxylate (4).

Solid NaH (1.5 eq as the oil dispersion) was added in small portions to a solution of pyrrole **3** (1 eq) and 3-bromopropyl phthalimide (1.2 eq) dissolved in DMF stirred at room temperature and flushed with argon. NOTE - Some gas evolves, but the temperature does not seem to rise above 40-50 °C. The reaction was stirred for 1.5 h at room temperature under argon. The reaction was followed by TLC (CH_2Cl_2/acetonitrile (95:5 v/v), the UV active product was at $R_f = 0.5$) and LCMS. Upon completion, the reaction was quenched with sat. aq. NH_4Cl (add slowly to avoid strong gas evolution and exotherm). Sat. aq. $NaHCO_3$ was then added to avoid an emulsion, and the basic organic mixture was extracted with ether. The combined ether layers were washed with sat. aq. $NaHCO_3$, water, brine, dried Na_2SO_4, filtered, and concentrated to dryness to give the crude product. The crude product was purified by eluting through silica with EtOAc/Hexane (1:4 v/v). The purified product contained some residual 3-bromopropyl phthalimide, that did not interfere with subsequent synthetic steps. The material was taken on and used without further purification. Assume a quantitative yield.

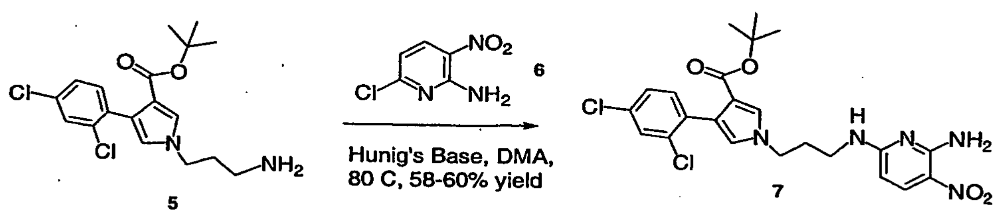


4 5

Preparation of *tert*-butyl 1-(3-aminopropyl)-4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (5).

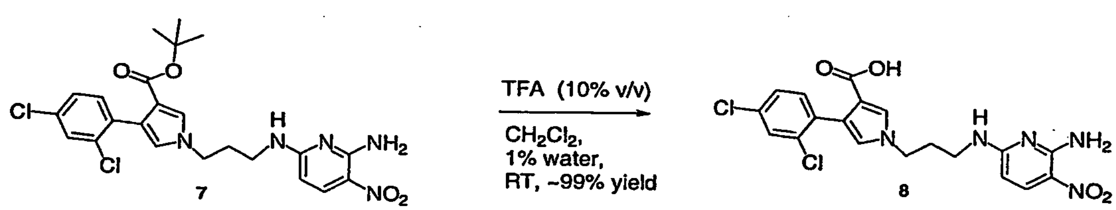
The Pthalimido Pyrrole **4** (1 eq) was dissolved in ethanol and hydrazine (3 eq) at room temperature under nitrogen. Upon heating to reflux, the reaction generated a white precipitate. Stir at reflux until complete (~2 h) by TLC (CH_2Cl_2/acetonitrile (95:5 v/v), the UV active product was at $R_f = 0.2$) and LCMS. Upon reaching completion, the reaction was allowed to cool to room temperature and the precipitate was vacuum-filtered off using a medium to fine cintered-glass filter. The filtrate was concentrated under reduced pressure to a gummy solid.

The crude material was taken up in ethanol/EtOAc (1:1 v/v), stirred and the precipitate was filtered off in the same fashion as before. The filtrate was concentrated under reduced pressure and than dried *in vacuo* for 10-15 min. This process of adding ethanol/EtOAc, filtering and concentrating was done one more time or as needed to remove the majority of the white precipitate and residual hydrazine. The product was then dried *in vacuo* overnight. The material was used without further purification. Once dried, the reaction yielded the product as a glass (~87% yield over 2 steps).



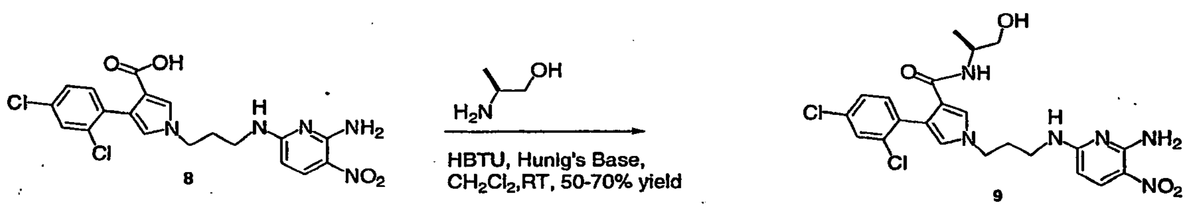
Preparation of *tert*-butyl 1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrole-3-carboxylate (7).

To the premixed dry reagents, pyrrole **5** (1 eq) and powdered 6-chloro-3-nitro-2-pyridylamine (**6**) (1.1 eq), was added the DMA followed by Hünig's base (2 eq) sequentially with stirring at rt. The reaction was then heated to 80 °C overnight. The reaction was followed by TLC (EtOAc/hexane (1:1 v/v), the UV active yellow product was at $R_f = 0.25$), HPLC and LCMS. Upon completion as judged by HPLC, the reaction was allowed to cool to 70 °C. Ethylene diamine (anhydrous) was then added to the reaction to destroy any remaining unreacted chloropyridine **6**. After 15 min stirring at 70 °C, the reaction was cooled and quenched with the addition of sat. aq. $NaHCO_3$. The aqueous mixture was extracted with EtOAc, and the combined organic layers were washed with sat. aq. $NaHCO_3$, water, brine, dried, filtered, and concentrated to dryness to give the crude product as a brown-yellow solid. The crude product was purified by flash chromatography eluted with EtOAc/hexane (4:6 v/v). The purified SnAr adduct **7** was isolated in 58% yield as a yellow solid.



Preparation of 1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrole-3-carboxylic acid (8).

In a vial, TFA (catalytic amount) was added to a stirred mixture of *tert*-butyl ester pyrrole 7 (1 eq), water (.1%), and CH_2Cl_2 at rt. The vial stirred at room temperature until done (~12 h. The reaction was then concentrated under reduced pressure at room temperature and dried *in vacuo*. The crude residue was dissolved again in CH_2Cl_2 and concentrated under reduced pressure at rt. The material was used in the final coupling step without further purification as the TFA salt.



Preparation of *N*-((1S)-2-hydroxy-isopropyl)(1-{3-[(6-amino-5-nitro(2-pyridyl))amino]propyl}-4-(2,4-dichlorophenyl)pyrrol-3-yl)carboxamide (9,).

(2S)-(+)-2-Aminopropan-1-ol (1.5 eq) was added to a stirred mixture of acid (**8**) (1 eq), HBTU (1.5 eq), Hünig's base (2 eq) and DMF (premixed sequentially in this order in a vial) at room temperature under argon. The reaction was stirred for 3-4 h until complete as shown by LCMS and HPLC. The reaction mixture was subsequently diluted with EtOAc, washed with $NaHCO_3$, and concentrated to afford a powder in a 70% yield.

Nomenclature for the Example compounds was provided using ACD Name version 5.07 software (November 14, 2001) available from Advanced Chemistry Development, Inc. Some of the compounds and starting materials were named using standard IUPAC nomenclature.

The compounds of Table 34 were synthesized following the synthetic methodology described above in the Examples and Schemes, and screened following methods 1 and 2 below. The precursors are readily recognizable by one skilled in the art and are commercially available from Aldrich (Milwaukee, WI) or Acros Organics (Pittsburgh, PA), among others.

Screening methods for SMIP/SMIS compounds

Method 1

Candidate small molecule immuno-potentiators can be identified *in vitro*. Compounds are screened *in vitro* for their ability to activate immune cells. One marker of such activation is the induction of cytokine production, for example TNF-α production. Apoptosis inducing small

molecules may be identified having this activity. These small molecule immuno-potentiators have potential utility as adjuvants and immuno-therapeutics.

In an assay procedure (High Throughput Screening (HTS)) for small molecule immune potentiators (SMIPs), human peripheral blood mononuclear cells (PBMC), 500,000 per mL in RPMI 1640 medium with 10% FCS, were distributed in 96 well plates (100,000 per well) already containing 5μM of compound in DMSO. The PBMCs were incubated for 18 h at 37°C in 5% CO_2. Their ability to produce cytokines in response to the small molecule compounds is determined using a modified sandwich ELISA.

Briefly supernatants from the PBMC cultures were assayed for secreted TNF using a primary plate bound antibody for capture followed by a secondary biotinylated anti-TNF antibody forming a sandwich. The biotinylated second antibody was then detected using streptavidin-Europium and the amount of bound europium was determined by time resolved fluorescence. SMIP compounds were confirmed by their TNF inducing activity that was measured in the assay as increased Europim counts over cells incubated in RPMI medium alone. "Hits" were selected based on their TNF-inducing activity relative to an optimal dose of lipopolysaccaride LPS (1 μg/ml), a strong TNF inducer. The robustness of the assay and low backgrounds allowed for the routine selection of hits with ~10% of LPS activity that was normally between 5-10X background (cells alone). Selected hits are then subjected to confirmation for their ability to induce cytokines from multiple donors at decreasing concentrations. Those compounds with consistent activity at or below 5μM are considered confirmed for the purposes of this assay. The assay is readily modified for screening for compounds effective at higher or lower concentrations.

Method 2

Each of the compounds in the above Table 34 elicited TNF-α production in human peripheral blood mononuclear cells. Many of the compounds showed activity at less than 20 μM with respect to production of TNF-α. Many of these compounds showed activity at less than 5 μM with respect to production of TNF-α. Many of these compounds showed activity in the production of TNF-α at less than 1.5 μM.

For this reason, each of the R groups of any of the compounds listed in Table 34 are preferred. Additionally, because of the excellent activity of each of the compounds, each of these compounds is individually preferred and is preferred as a member of a group that includes any or all of the other compounds and each compound is preferred in methods of modulating

immunopotentiation and in methods of treating biological conditions associated therewith, for example to be used as a vaccine adjuvant. Each of the compounds is also preferred for use in preparation of medicaments for vaccines, immunopotentiation, reducing tumor growth and in treating biological conditions mediated therefrom.

In additon to the procedure described above, methods of measuring other cytokines (e.g. IL1-beta, IL-12, IL-6, IFN-gamma, IL-10 etc.) are well known in the art and can be used to find active SMIP compounds of the present invention.

Compounds may be useful that cause production of TNF-α at higher concentrations, such as 100μM, 200 μM or 300μM in the assays described herein. For example Loxoribine causes useful production of TNF-α at 300μM (see Pope et al Immunostimulatory Compound 7-Allyl-8-Oxoguanosine (Loxoribine) Induces a Distinct Subset of Murine Cytokines Cellular Immunology 162: 333-339 (1995)).

The subject invention also includes isotopically-labeled antiviral compounds, that are structurally identical to those disclosed above, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into antiviral compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Antiviral compounds of the present invention, derivatives thereof, and pharmaceutically acceptable salts of said compounds and of said derivatives that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labeled antiviral compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, *i.e.*, ^{3}H, and carbon-14, *i.e.*, ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, *i.e.*, ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled antiviral compounds of this invention and derivatives thereof can generally be prepared by carrying out known or referenced procedures and by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

In accordance with the present invention, methods are provided for the administration of an effective amount of a SMIP compound to act as an adjuvant. Also provided are immunogenic compositions comprising a SMIP compound, an antigen, and optionally other adjuvants.

As adjuvants, the SMIP compounds are combined with antigens and delivery systems to form a final immunogenic composition or vaccine product.

As immunotherapeutics, the SMIP compounds are used alone or in combination with other therapies for treatment of SARS.

Those of ordinary skill in the art will recognize that physiologically active antiviral compounds, SMIPs or SMISs that have accessible hydroxy groups are frequently administered in the form of pharmaceutically acceptable esters. The antiviral compounds of this invention can be effectively administered as an ester, formed on the hydroxy groups, just as one skilled in pharmaceutical chemistry would expect. It is possible, as has long been known in pharmaceutical chemistry, to adjust the rate or duration of action of the antiviral compound by appropriate choices of ester groups.

Other compounds that can be used in combination with the therapeutic agents described herein include, pentoxifylline (PTX), methylprednisolone, trimetrexate (Neutrexin), Zadaxin (thymosin alpha 1), optionally substituted 5-aminomethinimino-3-methyl-4-isoxazolecarboxylic acid phenylamides, cyclosporine A (CsA), 6-oxo-1,4,5-thiadiazin[2,3-*b*]quinazoline, 3-amino-2(1*H*)-thioxo-4(3*H*)-quinazolinone, gangciclovir, glycyrrhizin, tetracyclines, aminoglycosides, quinolones, bicyclam (1,4-Bis(1,4,8,11-tetraazacyclotetradec-1-ylmethyl)benzene octahydrochloride dihydrate), rapamycin, wortmannin, enalapril, roquinimex/linomide, inactivin, DNCB, AG7088, 9-aminocamptothecin (CPT-11) , loxorobine, bropirimine, Ononase ® (ranpirnase), statins, such as: lovastatin--Mevacor®, pravastatin--Pravachol®, simvastatin--Zocor®, fluvastatin--Lescol®, atorvastatin—Lipitor® and rosuvastatin--Crestor®.

As used herein, the term "effective amount" means an amount of antiviral compound of the compositions, kits and methods of the present invention that is capable of treating the symptoms of the described conditions. The specific dose of a compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case including, for example, the compound administered, the route of administration, the state of being of the patient, and the severity of the condition being treated.

The dose of an antiviral compound of this invention to be administered to a subject is rather widely variable and subject to the judgment of the attending physician. It should be noted that it may be necessary to adjust the dose of a compound when it is administered in the form of a salt, such as a laureate, the salt forming moiety of which has an appreciable molecular weight.

The following dosage amounts and other dosage amounts set forth elsewhere in this description are for an average human subject having a weight of about 65 kg to about 70 kg. The skilled practitioner will readily be able to determine the dosage amount required for a subject whose weight falls outside the 65 kg to 70 kg range, based upon the medical history of the

subject and the presence of diseases, *e.g.*, diabetes, in the subject. Calculation of the dosage amount for other forms of the free base form such as salts or hydrates is easily accomplished by performing a simple ratio relative to the molecular weights of the species involved.

In general, the pharmaceutical compositions will include at least one antiviral compound in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water, borate-buffered saline containing trace metals or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, lubricants, fillers, stabilizers, *etc.* Methods of formulation are well known in the art and are disclosed, for example, in "Remington's Pharmaceutical Sciences," Mack Pub. Co., New Jersey (1991) or "Remington: The Science and Practice of Pharmacy," 20th ed., Lippincott Williams & Wilkins, Baltimore, Maryland (2000), incorporated herein by reference.

Pharmaceutical compositions for use within the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art.

Many of the active ingredient antiviral compounds are known to be absorbed from the alimentary tract, and so it is usually preferred to administer a compound orally for reasons of convenience. However, the compounds may equally effectively be administered intravenously, subcutaneously, percutaneously, or as suppositories for absorption by the rectum or vagina, if desired in a given instance. All of the usual types of compositions may be used, including tablets, chewable tablets, capsules, solutions, parenteral solutions, troches, suppositories and suspensions. Compositions are formulated to contain a daily dose, or a convenient fraction of daily dose, in a dosage unit, that may be a single tablet or capsule or convenient volume of a liquid.

Capsules are prepared by mixing the compound or compounds with a suitable diluent and filling the proper amount of the mixture in capsules. The usual diluents include inert powdered substances such as starch of many different kinds, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders.

Tablets are prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as the compound or compounds. Typical diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Typical tablet binders are substances such as starch, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose,

polyvinylpyrrolidine and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders.

A lubricant is generally necessary in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant is chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

Tablet disintegrators are substances that swell when wetted to break up the tablet and release the compound or compounds. They include starches, clays, celluloses, algins and gums, more particularly, corn and potato starches, methylcellulose, agar, bentonite, wood cellulose, powdered natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp and carboxymethylcellulose, for example, may be used as well as sodium lauryl sulfate.

Tablets are often coated with sugar as a flavor and sealant, or with film-forming protecting agents to modify the dissolution properties of the tablet. The compounds may also be formulated as chewable tablets, by using relatively large amounts of pleasant-tasting substances such as mannitol in the formulation, as is now well-established in the art.

When it is desired to administer a compound as a suppository, the typical bases may be used. Cocoa butter is a traditional suppository base, that may be modified by addition of waxes to raise its melting point slightly. Water-miscible suppository bases comprising, particularly, polyethylene glycols of various molecular weights are in wide use.

The effect of the compounds may be delayed or prolonged by proper formulation. For example, a slowly soluble pellet of the compound may be prepared and incorporated in a tablet or capsule. The technique may be improved by making pellets of several different dissolution rates and filling capsules with a mixture of the pellets. Tablets or capsules may be coated with a film that resists dissolution for a predictable period of time. Even the parenteral preparations may be made long-acting by dissolving or suspending the compound or compounds in oily or emulsified vehicles that allow dispersion slowly in the serum.

The combinations of this invention may be administered in a controlled release formulation such as a slow release or a fast release formulation. Such controlled release formulations of the combination of this invention may be prepared using methods well known to those skilled in the art. The method of administration will be determined by the attendant physician or other person skilled in the art after an evaluation of the subject's condition and requirements.

The term "prodrug" means compounds that are transformed *in vivo* to yield an antiviral compound of the present invention. The transformation may occur by various mechanisms, such as through hydrolysis in blood. A good discussion of the use of prodrugs is provided by T. Higuchi and W. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987. The term, "prodrug" also

encompasses mutual prodrugs in which one or more antiviral compounds are combined in a single molecule that may then undergo transformation to yield the individual antiviral compounds of the present invention.

For example, if an antiviral compound of the present invention contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a group such as (C_1-C_8)alkyl, (C_2-C_{12})alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxycarbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C_1-C_2)alkylamino(C_2-C_3)alkyl (such as β-dimethylaminoethyl), carbamoyl-(C_1-C_2)alkyl, N,N-di(C_1-C_2)alkylcarbamoyl-(C_1-C_2)alkyl and piperidino-, pyrrolidino- or morpholino(C_2-C_3)alkyl.

Similarly, if an antiviral compound of the present invention comprises an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as (C_1-C_6)alkanoyloxymethyl, 1-((C_1-C_6)alkanoyloxy)ethyl, 1-methyl-1-((C_1-C_6)alkanoyloxy)ethyl, (C_1-C_6)alkoxycarbonyloxymethyl, N-(C_1-C_6)alkoxycarbonylaminomethyl, succinoyl, (C_1-C_6)alkanoyl, α-amino(C_1-C_4)alkanoyl, arylacyl and α-aminoacyl, or α-aminoacyl-α-aminoacyl, where each α-aminoacyl group is independently selected from the naturally occurring L-amino acids, $P(O)(OH)_2$, $-P(O)(O(C_1-C_6)alkyl)_2$ or glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate).

If an antiviral compound of the present invention comprises an amine functional group, a prodrug can be formed by the replacement of a hydrogen atom in the amine group with a group such as R^X-carbonyl, R^XO-carbonyl, $NR^XR^{X'}$-carbonyl where R^X and $R^{X'}$ are each independently ((C_1-C_{10})alkyl, (C_3-C_7)cycloalkyl, benzyl, or R^X-carbonyl is a natural α-aminoacyl or natural α-aminoacyl-natural α-aminoacyl, $-C(OH)C(O)OY^X$ wherein (Y^X is H, (C_1-C_6)alkyl or benzyl), $-C(OY^{X0})\ Y^{X1}$ wherein Y^{X0} is (C_1-C_4) alkyl and Y^{X1} is ((C_1-C_6)alkyl, carboxy(C_1-C_6)alkyl, amino(C_1-C_4)alkyl or mono-N- or di-N,N-(C_1-C_6)alkylaminoalkyl, $-C(Y^{X2})\ Y^{X3}$ wherein Y^{X2} is H or methyl and Y^{X3} is mono-N- or di-N,N-(C_1-C_6)alkylamino, morpholino, piperidin-1-yl or pyrrolidin-1-yl.

The compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. Antiviral, SMIP, SMIS, or other immunomodulating compounds are prepared or obtained as described herein and in the US Patents and published international patent applications listed in

Table 1, Table 2, Table 34 and Table 35. The antiviral compounds can be formulated in pharmaceutically acceptable compositions suitable for delivery to the lungs. Particular formulations include dry powders, liquid solutions or suspensions suitable for nebulization and propellant formulations suitable for use in metered dose inhalers. The preparation of such formulations is well know to those skilled in the art, and is described in US Patent Nos. 5,814,607 and 5,654,007 and in the US Patents and published international patent applications listed in Table 3 the disclosures of which are incorporated herein by reference.

Dry powder formulations will comprise an antiviral compound in a dry, optionally lyophilized form with a particle size within a preferred range for deposition within the lung. Typically the particle size for deposition in the lung will range between 1 and 5 μm. When systemic delivery of the antiviral compound via absorption from the lung into the bloodstream is desired the antiviral compound formulation particle size is generally between 0.1 and 2 μm in size. The preferred size range of particles can be produced using methods such as jet-milling, spray drying and solvent precipitation, for example. Dry powder devices typically require a powder mass in the range from about 1 mg to 100 mg to produce an aerosolized dose. Thus, the antiviral compound will typically be combined with a pharmaceutically acceptable dry bulking powder. Preferred dry bulking powders include sucrose, lactose, trehalose, human serum albumin (HSA), phospholipids and glycine as well as those disclosed in the documents listed in Table 3. Dry powders can be administered to the subject in conventional dry powder inhalers. For liquid formulations the antiviral compound can be dissolved in any recognized physiologically acceptable carrier for use in delivery of aerosolized formulations. Such carriers include buffered and unbuffered aqueous solutions for water soluble compounds, and physiological solutions including saline solution (preferably between 0.2 and 2 N NaCl). For antiviral compounds with limited solubility, other liquid vehicles such as ethanol, propylene glycol and ethanol-propylene combinations may be used. The antiviral compounds may also be administered as solids in suspension.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray administered via pressurized packs or a nebulizer, with the use of a propellant, *e.g.*, air, dichlorordifluoromethane, dichloroterafluoroethane or other suitable gas. Preferably, for incorporation into the aerosol propellant, the antiviral compound formulations of the present invention will be processed into respirable particles as described above for the dry powder formulations. The particles are then suspended in the propellant, optionally being coated with a surfactant to enhance their disbursement. In the use of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Commercially available jet nebulizers are available and may be used to deliver aerosolized antiviral compound to a subject. Such jet nebulizers include, but are not limited to, those supplied by AeroTech 11 (CIS-US, Bedford, Mass.). In addition, for delivery of aerosolized antiviral compound to the lungs of a subject an oxygen source can be attached to the nebulizer providing a flow rate of, for example, 10 L/min. In general, inhalation is performed over a 5-40 minute time interval through a mouthpiece during spontaneous respiration. The present invention provides for novel compositions comprising a suitable carrier and aerosolized antiviral compound in doses sufficient to reduce or ameliorate viral load and SARS symptoms in subjects having SARS. Such doses can be lower than corresponding systemic doses that may be used to those generally used to reduce or ameliorate viral load and SARS symptoms in subjects having SARS.

The antiviral, SMIP, SMIS, and immunomodulating compositions of the present invention may be administered with a steroidal anti-inflammatory drug for the treatment of SARS and SARS symptoms. Examples of steroidal anti-inflammatory drugs of the invention include hydrocortisone, prednisolone, dexamethasone, triamcinolone acetonide, fluocinolone acetonide, fludrocortisone acetate, betamethasone, *etc.*

The antiviral compound composition of the invention is nebulized predominantly into particle sizes allowing a delivery of the drug into the terminal and respiratory bronchioles. For efficacious delivery of antiviral compound to the lung endobronchial space of airways in an aerosol, the formation of aerosol particles having mass medium average diameter predominantly between 1 to 5 μm is necessary. The formulation must additionally provide conditions that would not adversely affect the functionality of the airways. Consequently, the formulation must contain enough of the drug formulated under the conditions that allow its efficacious delivery while avoiding undesirable reaction.

For liquid solutions and suspensions, the choice of the nebulizer is made from among commercially available nebulizers. The jet nebulizers known as Sidestream O, obtained from Medicaid and Pari LCS, LC Plus, and eFlow obtained from Pari Respiratory Equipment, Richmond, Virginia, are examples of typical nebulizers suitable for the practice of the invention. Ultrasonic nebulizers that produce appropriate particle sizes of about 1 to 5 μm such as Aerosonic by DeVilbiss and UltraAire by Omron are also suitable.

Advantageously, the present invention also provides for a kit for use by a consumer for the treatment and/or prevention of SARS. Such a kit comprises: (a) a pharmaceutical composition comprising a therapeutically effective amount of at least one compound from among those described herein or listed in Table 34 and Table 35 or described in the US Patents and published international patent applications listed in Table 1, Table 2, and Table 35 and a pharmaceutically acceptable carrier, vehicle or diluent; (b) a container for holding the pharmaceutical composition;

and, optionally, (c) instructions describing a method of using the pharmaceutical compositions for the treatment and or the prevention of SARS. The kit may optionally contain a plurality of antiviral compounds for the treatment of SARS wherein the anti viral compounds are selected from 3C-like protease inhibitors and papain-like protease inhibitors. In a further embodiment, the kit contains an antiviral compound which is an RNA-dependent RNA polymerase inhibitor. When the kit comprises more than one antiviral compound, the antiviral compounds contained in the kit may be optionally combined in the same pharmaceutical composition.

A "kit" as used in the instant application includes a container for containing the separate compositions such as a divided bottle or a divided foil packet. The container can be in any conventional shape or form as known in the art that is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle that is in turn contained within a box.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil that is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It maybe desirable to provide a written memory aid, where the written memory aid is of the type containing information and/or instructions for the physician, pharmacist or subject, *e.g.*, in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested or a card that contains the same type of information. Another example of such a memory aid is a calendar

printed on the card *e.g.*, as follows "First Week, Monday, Tuesday," . . . *etc* . . . . "Second Week, Monday, Tuesday, . . ." *etc.* Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day. Also a daily dose of one or more component(s) of the kit can consist of one tablet or capsule while a daily dose of another one or more component(s) of the kit can consist of several tablets or capsules.

Another specific embodiment of a kit is a dispenser designed to dispense the daily doses one at a time in the order of their intended use. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter that indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

EXAMPLES

Example 1-EXAMPLE of a SARS VIRUS ISOLATE

A SARS virus was isolated from clinical specimens of a patient in Frankfurt, Germany (FRA). The isolate was grown in Vero cells. RNA of the SARS virus was extracted and amplified by RT-PCR. Nucleotide sequence of the viral genome was determined by direct sequencing of the PCR product. Computer analysis was used to predict the features of the genome, to compare it to previously known coronaviruses and to the sequence of different SARS virus isolates.

More specifically, isolation and sequence was performed as follows. After the third passage of the SARS virus in Vero cells, viral particles were purified by ultra centrifugation from $3x10^7$ cells supernatant. Viral RNA was extracted by Triazol method (Gibco-BRL). Viral RNA (200 ng) was transcribed into cDNA with avian RNaseH- thermostable reverse transcriptase following the instructions of the manufacturer (ThermoScript RT System, Invitrogen). Briefly, either 50 pmoles of oligo $(dT)_{20}$ (SEQ ID NO: 7389) or 25 ng of random hexamers were used to prime the RT reaction in a 20 μl final volume. Amplification and sequencing of the SARS genome were accomplished by direct sequencing of PCR products obtained with: i) specific primers from conserved regions of homology found through multiple alignment among known coronaviruses; ii) oligonucleotides designed around short sequences of SARS isolates available on the Web through WHO network laboratories; iii) degenerate primers to amplify the cDNA mixture with multiple overlapping fragments as end products. Gap closure

was realized by long distance PCR with high fidelity Taq (Expand High Fidelity system, Roche) using primers designed on selected fragments. Sequence was collected by primer walking using a BigDye terminator chemistry (Applied Biosystems) and an automated DNA sequencer (3700 capillary model, Applied Biosystems). After obtaining a first pass of the entire genome, a set of both forward and reverse primers were used to amplify and sequence *de novo* the genome using as a template DNA segments of 2 kb on average. Readings from overlapping fragments were automatically assembled by AutoAssembler (Applied Biosystems) and the 29,740 bp contiguous edited manually.

Computer analysis of the sequence was performed as follows. The GCG Wisconsin Package suite (version 10.0) was used for computer analysis of gene and protein sequences. The PSORT program (*http://psort.nibb.ac.jp/*) was used for localization predictions. For secondary structure analysis, the PHD software available on the Web at *http://cubic.bioc.columbia.edu/predictprotein/* was applied. The PSI-BLAST algorithm was used for homology searches (*http://www.ncbi.nlm.nih.gov/blast*) using the non-redundant protein database. ClustalW was applied to obtain multiple sequence alignments of gene and protein sequences. The LearnCoil-VMF program was used to predict coiled-coil regions in the spike proteins (*http://learncoil-vmf.lcs.mit.edu/cgi-bin/vmf*). Leucine zippers were predicted with the program 2ZIP, available at *http://2Zip.molgen.mpg.de*.

Phylogenetic analysis was performed using the neighbor-joining algorithm as implemented in the program NEIGHBOR within the Phylogeny Inference Package (Phylip) (Felsenstein J 1993, program distributed by the author). Bootstrap analysis was always performed with 100 replicates using the program Seqboot. Trees were handled and displayed using TreeView. The program HMMER was used to generate sequence profiles from multiple sequence alignments of the S1 domains of spike proteins. Subsequently, the HMMPFAM program was used to compare the S1 domain of SARS spike to the profiles.

The genome of this SARS virus isolate is 29,740 bases long and the overall structure of the genome is similar to that of the three known groups of coronaviruses. Starting from the 5' end a leader sequence, an untranslated region (UTR) and two overlapping open reading frames coding for one polyprotein containing the enzymes necessary for replication can be identified. They are followed by a region coding for the spike (S), envelope (E), matrix (M), nucleocapsid (N) structural proteins and eight additional ORFs specific for the SARS virus. At the 3'-end of the genome a UTR with a poly(A) is located. The overall homology to coronaviruses groups 1, 2 and 3 is low and therefore the SARS virus belongs to a new group (group 4) of coronavirus. More detailed analysis of the spike protein amino acid sequence shows that the SARS virus isolate is more closely related to coronavirus group 2.

The complete genome sequence of the SARS virus isolate is 29,740 bp in length. The sequence is available on Genbank and has a GC content of 40.8%, comparable with that of known viruses of the same family. Genome structure is similar to that of other coronaviruses. 14 open reading frames have been predicted. The principal features of the genome and gene products are illustrated reported in Figure 17 and Table 10. The comparison between the SARS genome and those of group 1, 2 and 3 coronaviruses is reported in Figure 18.

Nucleotides 1-73 contain a predicted RNA leader sequence followed by an untranslated region (UTR) of 197 nucleotides. The UTR is followed by two overlapping open reading frames (ORF1a, ORF1b), which encompass two-thirds of the genome (nucleotides 265-21485). They encode for a large polyprotein, which is predicted to be processed by viral proteases to generate the replicase complex. The 3' part of the genome contains the genes coding for the four structural proteins (S, spike protein, E, envelope protein, M, matrix glycoprotein, and N, nucleocapsid protein), and eight predicted ORFs of unknown function (Figure 17). Finally, at the 3' end of the genome, we found a second UTR of 340 bases followed by a poly(A) tract. We identified a putative intergenic (IG) sequence also referred to as transcription-associated sequence (TAS), which is a typical feature for coronaviruses. The IG sequence is characterized by 6-18 nucleotides present at the 3' end of the leader and can be found in front of each gene. The IG sequence plays a key role in RNA transcription and its regulation. The IG sequence of the SARS virus is characterized by the sequence SEQ ID NO: 7293 and is present nine times in the genome (Figure 17). The sequence of the leader and IG are peculiar for each coronavirus and represent a specific signature for the virus.

The Replicase Region

The replicase gene, ORF1ab (SEQ ID NO: 7232), consists of two overlapping ORFs, ORF1a and ORF1b, which can be translated as a single polyprotein by frame shift of the ribosome in position 13,393, within the polymerase encoding region. See Brierley et al, *Embo J* 1987: 6(12): 3779-3785. As expected, a stem-loop sequence is present ten base pairs downstream of this site (SEQ ID NO: 7390; 5'-CGGTGTAAGTGCAGCCCGTCTTACACCG-3'). The polyprotein is cleaved co- and/or post-translationally into multiple proteins by its own encoded proteases. Using the cleavage consensus sequence and by analogy with other coronaviruses, we have mapped the possible cleavage sites of the polyprotein and have identified 14 products, which comprise the leader protein p28, the homologue of the MHV p65 protein and other twelve proteins, named from nsp1 to nsp13 (nsp, non structural protein) (Figure 17 and Table 10). The amino acid sequence analysis suggests the presence of several functional motifs within the putative ORF1ab proteins. In particular, we have mapped two potential proteases (nsp1 and nsp2), one growth factor-like motif (nsp7) within ORF1a, whereas in ORF1b we identified the RNA polymerase (nsp9), and a

predicted helicase (nsp10). The other predicted cleavage products (nsp3, nsp4, nsp5, nsp6, nsp11, nsp12 and nsp13) are proteins of unknown function. Many of these proteins are presumably present in the RNA replication complex, which is associated with the membranous structures in the infected cells. In particular, nsp3 and nsp4 contain hydrophobic domains. As shown in Figure 18, the replicase region of SARS has a similar organization to group 1, 2 and 3 coronaviruses; however, the overall aminoacid conservation is low (Table 11). The most conserved proteins are the polymerase and the helices.

Nsp1 is the papain-like cysteine protease (PLP), which cleaves the first two protein products (leader protein p28 and p65 homologue). Within the nsp1 of MHV, two domains with papain-like protease activity (PLP1 and PLP2) have been mapped, (Kanjanahaluethai *et al* (2000) *J. Virol* 74(17):7911-21) which are also conserved with Bovine, transmittable gastroenteritis virus (TGV) and Human 229E coronaviruses. However, by sequence alignment with the SARS nsp1, we identified only one PLP domain containing the catalytic residues Cys833 and His994.

Nsp2 is the chymotrypsin-picornavirus 3C-like protease (3CLp), which is responsible for the post-translational processing of the other 12 proteins, most of them cleaved at Q/A or Q/S sites. (Ziebuhr *et al* (1999) *J. Virol* 73(1):177-85). It also performs autoproteolytic activity. The principal catalytic residues are well conserved with other coronaviruses and are located at position His41 and Cys145. Furthermore, even the conserved aminoacids Tyr161 and His163, which are believed to be involved in substrate recognition and to be indispensable for proteolytic activity, (Hegyi *et al* (2002) *J. Gen Virol* 83(Pt3):581-593) were found in the sequence of the SARS 3CLp.

The invention includes the orf1ab sequence of SEQ ID NO: 9960 and the orf1a sequence of SEQ ID NO: 9961, including fragments, variants, homologs, *etc.* thereof.

The Structural Region

Analysis of the nucleotide sequence at the 3' part of the SARS genome identified 12 predicted open reading frames. They are coded within 8.2 kb and comprise the four structural proteins S, E, M and N, common for all coronaviruses and eight predicted ORFs, which are specific for this virus (Figure 18). SARS-specific IG sequences upstream of most ORFs (Figures 17 & 18) suggest that most genes are likely to be transcribed independently. Interestingly, sequences identical to the group 2 IG are also present at the end of the RNA leader and in front of the Matrix encoding gene and of ORF 10.

The spike is a type I glycoprotein, which forms the large spikes on the surface of the virion and is responsible for receptor-binding and membrane fusion. (Gallagher (2001) *Adv Exp Med Biol* 494: 183-92). The protein is 1255 residues long with 17 predicted N-glycosylation sites. It has a 13aa leader peptide and a 17 aa C-terminal membrane anchoring sequence (1202-1218).

Some (MHV, HCoV-OC43, AIBV and BCoV), but not all (TGV, FIPV, HCoV-229E) coronavirus spike proteins are proteolytically cleaved in two subunits, S1 and S2. S1 is supposed to form the bulbous head, which stays non-covalently linked to the C-terminal membrane anchor. Cleavage is mediated by a basic aminoacid sequence, which resembles the consensus sequence for a furin cleavage site. (Garten *et.al.*, *Biochimie* 1994; 76(3-4): 217-225). However, in case of this SARS virus isolate, we were not able to identify such a sequence, implicating that the S protein of this SARS virus isolate is unlikely to be cleaved during maturation. Secondary structure predictions indicated that the global architecture of the spike protein is conserved within all known coronaviruses. The S1 domain is mainly formed by beta sheets and likely adopts a globular fold, while in the S2 domain extensive alpha helical regions are predicted. In addition, the LearnCoil-VMF program, specifically designed to identify coiled-coil-like regions in viral membrane-fusion proteins, predicts two coiled-coils within S2, spanning aminoacids 900-1005 and 1151-1185, respectively (Figure 19). Both coiled-coil regions contain a leucine-zipper motif, which is also present in the spikes of all coronaviruses. Leucine zippers are known to promote protein oligomerization; since the spike proteins of TGV and MHV form hetero-trimers, (Delmas *et al*, *J Virol* 1990; 64(11):5367-5375) (Godeke, *et al.*, J *Virology* 2000; 74(3):1566-1571) it is conceivable that in SARS leucine zippers play a role in promoting and/or stabilizing a similar quaternary structure. The spike protein plays a major role in the biology of coronaviruses because the S1 domain contains the receptor-binding domain and the virus neutralizing epitopes, while the S2 domain is involved in the process of membrane fusion, which is essential for virus infectivity. As expected, multiple sequence alignment of different spike proteins showed a major degree of variability within the S1 domain, whereas S2 is more conserved.

The envelope protein E is a very short polypeptide of 76 aa, involved in the morphogenesis of the virion envelope. (Godet *et al.*, *Virology* 1992; 188(2):666-675). Computer analysis predicts a long transmembrane domain close to the N-terminus and two N-glycosylation sites. The level of aminoacid similarity with other coronaviruses is very low and the best homology is with the small envelope protein of the transmissible gastroenteritis virus (TGV).

The matrix glycoprotein (M) is a 221-residue polypeptide with a predicted molecular weight of 25 kDa. Computer analysis predicts a topology consisting of a short aminoterminal ectodomain, three transmembrane segments and a carboxyl terminus located at the interior side of the viral envelope. In analogy with the matrix glycoprotein of TGV, that of the avian infective bronchitis virus (AIBV) and that of the hypervirulent MHV-2 strain the SARS M glycoprotein is N-glycosylated at the N-terminus. SARS M protein shows highest similarity to group 2 viruses (Table 11).

Finally, the nucleocapsid protein N is a 397-residue-long phosphoprotein that interacts with viral genomic RNA to form the nucleocapsid. The level of conservation with other coronaviruses is low, ranging from 26,9% identity with the HCoV-229E to 37,4% identity to the Bovine coronavirus (BcoV) (Table 11). Epitope analysis of the nucleocapsid protein has been carried out (Li *et al.* (2003) *Geno Prot & Bioinfo* 1:198-206) in which the epitope site at the C terminus of the protein was located as SEQ ID NO: 7394 (amino acids 371-407 of SEQ ID NO: 6052).

In addition to the above fundamental proteins, many viruses express a set of other peptides, which are generally dispensable for viability, but can influence the infectivity potential of the virus. (de Haan *et al.*, Virology 2002; 296(1):177-189). These proteins are generally conserved within members of the same serogroup, but differ profoundly among the groups. For this reason, they are generally referred to as group-specific proteins (Figure 11). Members of the group 1, represented here by HcoV-229E, have two group-specific genes located between the S and E genes and sometimes one or two ORFs downstream of the N gene, preceding the 3' UTR region of the genome. Viruses of the group 2, with MHV as prototype, have two group-specific genes (2a and HE) between ORF1b and S, as well as other two between S and E genes. Finally, the group 3 viruses, represented by the prototype AIBV, have two group-specific genes between S and E and other two between the M and N genes.

With the exception of the hemagglutinin esterase HE, for which hemagglutinating and acetyl-esterase enzymatic activities have been demonstrated, all the other group-specific ORFs encode proteins whose role has not yet been established.

Interestingly, the arrangement of specific genes in the SARS genome is peculiar and the predicted ORFs do not display any significant homology with ORFs present in the other coronaviruses, nor with any other known protein from different organisms. Like viruses of the group 1 and 3, SARS lacks the HE hemagglutinin and does not contain ORFs between the ORF1b and the S gene. Furthermore, two predicted ORFs (ORF3 and ORF4) are encoded in the region between S and E, and superimpose for most of their length. ORF3 has an IG sequence 2 bp upstream of the ATG start codon. In contrast to the other groups, SARS contains five predicted ORFs in the region between M and N genes. ORF7 is located 10 bases downstream of the stop codon of M gene, and has an IG sequence 155 nucleotides upstream from the ATG start codon. Similarly, ORF8 and ORF10 present an IG right upstream of their ATG start codons. On the other hand, the 5' ends of ORF9 and ORF11 shortly superimpose with the flanking genes, and for this reason they do not need an IG to activate transcription. ORF12 totally superimposes with the N gene and shares very low homology with a 22kDa protein of the MHV virus, coded in the corresponding region.

Despite the absence of indications of possible localization and function deriving from sequence similarity, ORF3, ORF7 and ORF8 contain hydrophobic segments, suggesting association with membrane structures. In addition, ORF3, the longest among the SARS specific gene, is the only one that encodes for a peptide containing a high number of predicted O-glycosylation sites (Table 11). Predicted N-glycosylation sites have been identified in ORF3, ORF11 and ORF12.

Two shorter ORFs in the non-structural regions are SEQ ID NOS: 9965 and 9966. The invention includes polypeptides with these sequences, and also fragments, variants, *etc.*

Phylogenetic analysis

The substitution frequency within 922 conserved bases from the *pol* gene of eleven coronaviruses from the three different serogroups has been used in the past to show that the variability within members of each serogroup is much smaller than between members of different serogroups, confirming the previously described serological groupings. (Stephensen *et al.*, Virus Res 1999; 60(2):181-9). We used the 922 bp region of the *pol* gene of SARS and aligned it with the same fragment from other 12 coronaviruses. The tree obtained showed that the SARS virus is distinct from the other three groups of coronaviruses (Figure 20). Similar results were obtained using the full-length aminoacid sequences of *pol*, 3CL-protease and helicase from the replicase region and those of the spike and the matrix glycoproteins from the structural region (data not shown). These data confirmed that the entire genome of the SARS virus clusters in a new group (group 4) of coronavirus.

To gain more resolution for possible evolutionary relationships we performed the analysis using consensus sequences of predicted domains of the proteins. In particular, we generated consensus sequences of the S1 domain of the spike protein from the group 1 and group 2 and then we compared them to the S1 domain of the SARS spike. No consensus could be generated from the group 3 since only the spike protein of AIBV is known. Interestingly, the tree constructed from the alignment of SARS S1 with the consensus generated from the two groups of spike proteins was different from that in Figure 20, and showed a much closer relationship between SARS and group 2 coronaviruses (Figure 21A). Further analysis showed that 19 out of the 20 cysteines present in the SARS S1 domain are spatially conserved with the group 2 consensus sequence, while only five are maintained either within the group 1 and group 3 sequences (Figure 21B). Given the fundamental role played by cysteines in protein folding, it is likely that the S1 domain of SARS and group 2 coronaviruses share a similar spatial organization.

Sequence variability between SARS coronaviruses

We compared the FRA sequence to the four complete SARS genomes available on the Web. A total of 30 mutations were detected. Nine of these mutations were silent while 21 resulted in aminoacid substitutions (Table 12). Within ORF1a, three silent and seven productive mutations were detected. In ORF1b, there were five silent and three productive mutations. One of the productive mutations was caused by two nucleotide substitutions resulting in a single aminoacid change. Five changes were located in the spike protein, four of these were productive and one silent. Two productive mutations were in ORF3 and in the matrix glycoprotein M. One productive mutation each was in ORF10 and in the nucleocapsid protein N.

The overall difference between FRA and TOR2 was of nine nucleotides resulting in two silent mutations and seven aminoacid changes. The difference between FRA and Urbani is 12 nucleotides, which result in five silent mutations and seven aminoacid changes. For CUHK 16 nucleotides were different, five of which were silent mutations. For FRA and HKU 14 nucleotide changes resulted in four silent and nine productive mutations.

EXAMPLE 2 -Production, Inactivation and Purification of Whole SARS Virus Using MCS Chromatography Resin Purification Followed by Density Gradient Ultracentrifugation

A SARS isolate FRA1 (EMBL: AY310120) was passaged on VERO cells that were cultivated in DMEM (Gibco: Cat No. 21969-035, Lot No. 3078864), Penicillin/Strep (Gibco: Cat No. 15070-063, Lot No. 1120042), and 3% FCS (Gibco: Cat No. 10270-106, Lot No. 40F6130K) at 37°C, 5% CO_2. Trypsin (Gibco: Cat No. 25300-054, Lot No. 3078729) was used for detaching the cells.

For virus production the third passage was used for inoculation of VERO cells at a moi of ~0.1. Cells were incubated with the virus for 1 h at 37°C in infection medium (DMEM without PS, FCS); after 1h cells were washed twice and further incubated at 37°C for 48 h in the presents of 3% FCS and antibiotics. The supernatant was harvested 48 hours post infection (p.i.) and precleared by centrifugation at 3000 rpm at 4°C for 10 min.

The SARS virus was inactivated by β-propiolactone (BPL) treatment (1:2000) for 18 h at 4°C, followed by 3 h at 37°C. Testing the virus on successful inactivation, VERO cells were incubated with 10 ml BPL treated supernatant for 4 days at 37°C; subsequentially, the supernatant was transferred to a fresh VERO cell culture and further incubated for another 4 days. Cells were checked for cytopathic effect (CPE).

200 ml of the BPL-inactivated SARS virus harvest was then clarified using a 0.65 μm-pore-size filter (47 mm diameter) to pass virus particles and retain cell debris. The filter unit was connected to a Masterflex pump, which accomplished a consistent flow rate of 40 ml/min.

A. MCS Chromatography Purification Step

The filtered virus suspension was then subjected to MCS chromatography. The MCS column was prepared as follows. 27 ml slurry led to 14 ml sedimentated resin which was packed using a Götec Superformance Column (diameter 1.0 cm, height 15.7 cm, volume 12.33 ml). 1% of the column volume of a 1% acetone solution was injected to the column and the column was run with a flow of 100 cm/h. The HETP, N and A_s values were then calculated as HETP: 0,056 cm, N / m: 1790 and $A_s = 1.20$.

The amount of proteins in the purified solution after the MCS chromatography step were assessed with a bicinchoninic acid (BCA) method (Interchim) (*see, e.g.*, *http://www.piercenet.com/files/bca.pdf*) and electrophoresis.

SDS-PAGE was done in accordance to Laemmli, *Nature* (1970) 227:680-685. Samples for SDS-PAGE were diluted to a protein concentration of 77 μg/ml. Different protein concentrations were loaded depending on the gel types used (10/12/15 Wells, Novex/Invitrogen):

Number of Wells	Protein Concentration in the Dilution	Load	Protein/Well
10 Wells	77 μg/ml	20 μl	1 μg
12 Wells	77 μg/ml	15-20 μl	0.75 - 1 μg
15 Wells	77 μg/ml	10 μl	0.5 μg

Samples for use in a reducing SDS-PAGE were prepared as follows:

	26 μl sample or diluted sample
	+ 10 μl NuPage Sample Buffer (4x) SDS NP0003
	+ 4 μl TCEP Bondbreaker Solution 77720 (1:2 in MilliQ water)
Final Volume:	40 μl

The samples were heated for 10 minutes at 70°C or left at room temperature for 1 hour (leaving the samples at room temperature prevents the M protein of Corona Virus to coagulate/forming complexes), and then centrifuged for approximately one minute at 14,000 rpm in a table top centrifuge.

Markers for use on the gel were prepared as follows. Gel bands containing less than 1 μg of proteins were easily visualised with the silver staining procedure using the Silver Staining Kit Protein, Plus One Staining Protocol (Pharmacia Biotech).

Western blotting was performed as follows. A semi-dry blotting technique was used to transfer the proteins from the SDS gel to a nitrocellulose membrane. The transfer was performed with a current of 0.8 mA/cm^2 for 1 hour. A rabbit polyclonal antibody against SARS virus was used to perform the immuno probing using the Western Breeze, Novex Chromogenic Western Blot Immunodetection Kit (Novex/Invitrogen).

The chromatogram of the inactivated SARS MCS capture step is depicted in FIGURE 27. To estimate purity, MCS chromatography fractions were analysed by silver staining on NuPage

10% or 4-12% Bis-Tris-Gel (Novex) under reduced conditions, heated for 10 minutes at 70°C (Figure 28). The fractions were also analysed under the same conditions by western blot (Figure 29) to estimate purity, using PAK 11/03 SARS Cov 270603 neutralizing titer 1:512 (this antibody was used for this and subsequent western blots). Purity estimates are as follows:

Sample	Volume / ml	[Protein] / μg/ml	Total Protein / mg	Step Recovery Protein / %
Corona Harvest	100	2547.6	254.76	100
After Filtration = Load	100	2440.3	244.03	95.8
Flow Through	85	2321.4	197.32	77.5
Wash	49.32	468.5	23.11	9.1
Peak 1	12.12	252.7	3.062	1.2
Total Recovery	-	-	464.4	86.5

B. Density Gradient Ultracentrifugation Step

The eluted SARS virus fraction was then subjected to density gradient ultracentrifugation with a swinging bucket rotor to further purify the inactivated virus. 3 ml of MCS peak fraction were loaded onto a linear gradient (15-60% sucrose; 17 ml 15% and 17 ml 60% sucrose in gradient mixer). The separation was performed with a Beckman SW 28 rotor at 20,000 rpm for 2 hours.

The content of sucrose and protein in the linear density gradient ultracentrifugation fractions are depicted in the following table, the graph in figure 30 and the estimation of purity in figure 31:

Fraction	Fraction Size / ml	[Sucrose] / %	[Protein] / μg/ml
1	2	61	96.12
2	2	59.4	98.62
3	2	57.5	87.63
4	2	54.5	86.91
5	2	50.5	79.9
6	2	47.2	74.3
7	2	43.7	68.05
8	2	40.2	60.43
9	2	37.2	57.38
10	2	34	53.12
11	2	30	50.63
12	2	25.7	35.02
13	2	22.4	35.33
14	2	19.5	39.25
15	2	15.5	69.79
16	2	8.5	169.03
17	2	8.5	128.96

The protein concentration of fraction 11 (Figure 31 SDS-gel) was measured again against a standard curve prepared in 30% sucrose and lead to a protein concentration of 3.67 μg/ml (0.05

μg on the gel). The M protein appears to be missing in this preparation possibily due to sample treatment procedure (heated samples).

There may be discrepancies in the protein concentration measurements in Table 2 due to sucrose interference with this assay.

EXAMPLE 3 -Production, Inactivation and Purification of Whole SARS Virus Using MCS Chromatography Resin Purification Followed by Density Gradient Ultracentrifugation

Inactivated SARS virus was prepared as described in Example above.

A. MCS Chromatography Purification Step

In this example, 200 ml of inactivated SARS virus harvest were subjected to MCS chromatography. The chromatogram of the capture step of inactivated SARS virus purification with MCS is depicted in FIGURE 32, the protein recovery in the following table and the estimation of purity in FIGURE 33:

Sample	Volume / ml	[Protein] / μg/ml	Total Protein / mg	Step Recovery Protein / %
Corona Virus Harvest	200	2239.2	447.83	100
After Filtration = Load	200	2245.1	449.02	100.3
Flow Through	185	2126.3	393.37	87.8
Wash	49.32	450.1	22.2	5.0
Peak 1	4.43	1245.6	5.52	1.2
Total Recovery	-		421.08	93.7

B. Density Gradient Ultracentrifugation Step

3.5 ml of MCS peak fraction were then loaded onto a linear gradient (15-40% sucrose: 16 ml 15% and 16ml 40% sucrose in gradient mixer). The separation was performed with a Beckman SW 28 rotor at 20,000 rpm for 2 hours.

The content of sucrose and protein in the linear density gradient ultracentrifugation fractions are depicted in the following table and the graph in FIGURE 34:

Tube	Fraction Size / ml	[Sucrose] / %	[Protein] / μg/ml
1	2	40	45.86
2	2	39	45.68
3	2	37.5	44.14
4	2	35.5	37.82
5	2	33.5	34.48
6	2	31.5	31.76
7	2	30.5	29.49
8	2	28	30.87
9	2	25.5	31.7
10	2	23.5	26.74
11	2	21.75	23.58
12	2	20	35.33
13	2	18	96.38
14	2	14.5	523.79

15	2	8	941.97
16	2	8	696.7

Protein recovery is shown in the following table and the estimation of purity is shown in figure 35. Electron Micrograph pictures of density gradient fractions 8, 9 and 10 are shown in figure 36:

Step	Volume / ml	Protein / μg/ml	Total Protein / mg	Step Protein %
Load	3.5 ml	1245.6	4359.6	100
Bulk Protein Fractions	3.5 ml	720.8	4324.9	99.2
Viral Peak Fraction	8 ml	29.7	237.6	5.5
Total Recovery			4562.5	104.7

EXAMPLE 4 - Mouse Immunization with Inactivated SARS Virus

Mice were immunized subcutaneously on days 0, 14, and 28 with 5 μg BPL-inactivated SARS-CoV particles (BPL-SARS-CoV), either alone or together with Alum or MF59 as adjuvants. Serum was collected on days 0 (pre-immunization), 13 (post 1st immunization), 28 (post 2nd), and 35 (1 week post 3rd immunization). Neutralizing antibodies were assessed for blocking SARS-CoV infection of Vero cells *in vitro*. After 3 immunizations, neutralization titers were in the range 1:100-1:1000, which are levels similar to those present in the serum of SARS convalescent patients. As shown in the following table, the non-adjuvanted vaccine induced neutralizing antibody after the third immunization, and potency of this vaccine was enhanced significantly by including the adjuvants, with neutralizing antibody appearing after then 2nd immunization and overall titers increasing after then 3rd immunization:

Immunogen	Neutralization Titer			
	pre	post 1st	post 2nd	post 3rd
BPL-SARS-CoV+MF59 (5 μg)	< 1:20	< 1:20	1:158	1:630
BPL-SARS-CoV+Alum (5 μg)	< 1:20	< 1:20	1:67	1:612
BPL-SARS-CoV (5 μg)	< 1:20	< 1:20	< 1:20	1:71
PBS	< 1:20	< 1:20	< 1:20	< 1:20

EXAMPLE 5 - Balb/cMouse Immunization with Inactivated SARS Virus

A Balb/c mouse model for SARS infection has been developed (Subbarao *et al.* (2004), *J.Virol.*, 78:3572-77. In this model, Balb/c mice are inoculated intranasally with 10^4 $TCID_{50}$ of virus. At 48 hours post-inoculation, a 2-log increase in the $TCID_{50}$ virus titer can be detected in the lungs of infected mice. While virus replication is readily detected, the mice do not show any SARS disease symptoms and spontaneously clear the virus one week after inoculation. A decrease in virus titer in previously-immunized animals as compared to control animals demonstrates a protective effect of the vaccine being evaluated.

In this example, four Balb/c mice per group are immunized three times with 5 μg BPL inactivated SARS-CoV (days 0, 14, 28) either alone or in combination with MF59 and

challenged with 10^4 $TCID_{50}$ of SARS-CoV on day 43. Two days following virus challenge the mice are euthanized and SARS-CoV is quantified from nasal turbinates (NT) and lungs and the mean virus titer for each mouse is measured. Control groups received PBS alone, or an influenza virus vaccine (FLU) with or without MF59 adjuvant. Data were as follows (see also Figure 51), where four mice were tested per group and virus titers are expressed as $\log_{10}$ $TCID_{50}$ per gram of tissue:

Immunogen	Virus replication in lungs of challenged mice		Virus replication in nasal turbinates of challenged mice	
	# infected/ # tested	Mean (± SE) virus titer	# infected/ # tested	Mean (± SE) virus titer
PBS	4/4	6.3 ± 0.3	3/4	2.8 ± 0.35
MF-59 alone	4/4	6.1 ± 0.13	3/4	3.0 ± 0.38
FLU vaccine (5 μg)	4/4	6.3 ± 0.07	3/4	2.9 ± 0.36
FLU vaccine (5 μg) + MF-59	4/4	6.0 ± 0.19	4/4	3.0 ± 0.11
BPL-SARS-CoV (5 μg)	¼	1.6 ± 0.13 *	0/4	Not detected **
BPL-SARS-CoV (5 μg) + MF-59	0/4	Not detected *	0/4	Not detected **

Two-tailed Student's t-test, compared to PBS-immunized mice, showed: * $P<0.00001$ or ** $P=0.025$

As shown, virus could not be detected in the BPL-SARS-CoV immunized mice. The lower limit of detection of infectious virus in a 10% w/v suspension of lung homogenate was 1.5 $\log_{10}TCID_{50}$/gm, and in a 5% w/v suspension of nasal turbinates the limit was 1.8 $\log_{10}TCID_{50}$/gm. Viral titers in the immunized mammals were thus below these threshold values.

Thus the inactivated SARS-CoV vaccine was very efficient at preventing virus infection, as only one of eight mice immunized with the vaccine, either with or without MF59 adjuvant, was infected. Similar protection was not observed in control groups of PBS diluent, MF59 adjuvant, or influenza virus vaccine with or without adjuvant.

Neutralization titers of sera taken from the animals in the challenge study were assessed at two weeks post-1st, one week post-2nd, and one week post-3rd immunization. Mice immunized with the vaccine with MF59 adjuvant had already developed a neutralization titer of 1:71 after the 2nd immunization, which increased to 1:588 after the 3rd immunization, whereas mice receiving the unadjuvanted vaccine did not have any neutralizing activity post-2nd and a neutralization titer of 1:64 post-3rd immunization. Sera from mice in each of the control groups did not show any neutralization activity. These data clearly demonstrate not only the ability of the inactivated SARS-CoV vaccine to induce protective levels of SARS neutralizing antibodies, but also a beneficial effect of formulating the vaccine with adjuvant for elevated neutralization titers.

EXAMPLE 6 - Preparation of OMV comprising SARS viral antigens

E.coli were transfected with a plasmid of interest (encoding a SARS viral antigen). Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp

(100 μg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium and grown at either 30°C or 37°C until the OD_{550} reached 0.6-0.8. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0 mM. After incubation for 3 hours, bacteria were harvested by centrifugation at 8 000 x g for 15 minutes at 4°C and resuspended in 20 ml of 20 mM Tris-HCl (pH 7.5) and complete protease inhibitors (Boehringer-Mannheim™). All subsequent procedures were performed at 4°C or on ice.

Cells were disrupted by sonication using a Branson Sonifier 450 and centrifuged at 5 000 x g for 20 min to sediment unbroken cells and inclusion bodies. The supernatant, containing membranes and cellular debris, was centrifuged at 50000g (Beckman Ti50, 29 000 rpm) for 75 min, washed with 20 mM Bis-tris propane (pH 6.5), 1.0 M NaCl, 10% (v/v) glycerol and sedimented again at 50000g for 75 minutes. The pellet was resuspended in 20mM Tris-HCl (pH 7.5), 2.0% (v/v) Sarkosyl, complete protease inhibitor (1.0 mM EDTA, final concentration) and incubated for 20 minutes to dissolve inner membrane. Cellular debris was pelleted by centrifugation at 5000g for 10 min and the supernatant centrifuged at 75000g for 75 minutes (Beckman Ti50, 33000 rpm). Outer membrane vesicles were washed with 20 mM Tris-HCl (pH 7.5) and centrifuged at 75 000 x g for 75 minutes or overnight. The OMV was finally resuspended in 500 μl of 20 mM Tris-HCl (pH 7.5), 10% v/v glycerol. Protein concentration was estimated by standard Bradford Assay (Bio-Rad), while protein concentration of inner membrane fraction was determined with the DC protein assay (Bio-Rad). Various fractions from the isolation procedure were assayed by SDS-PAGE.

EXAMPLE 7 - Immunogenicity, dose and route schedule for recombinant Spike protein in mice

The immunogenicity, route and dosing of the recombinant spike proteins of the invention in mice may be assessed using the below detailed protocol. Preferably, the administered antigen will elicit neutralizing antibody titers at least in the range of 1/100-1/1000. Increasing doses of antigen can be tested in the range from 5 to 20 μg of recombinant Spike antigen alone or mixed with an equal volume of MF59-citrate, administered SC or IM to anesthetized mice in 100 μl of inoculum. Groups of BALB/c mice, 6 per treatment are primed at day 0 and boosted at day 14 and 28.

Group	Treatment	Dose/Route	Sampling interval	Number of mice
1-3	Rec-Spike protein	20, 10, 5 μg/SC	7, 21, 35, 42 d	6 per dose level
4-6	Rec-Spike protein	20, 10, 5 μg/SC	7	6 per dose level
7-9	Rec-Spike protein	20, 10, 5 μg/IM	7, 21, 35, 42 d	6 per dose level
10-12	Rec-Spike protein	20, 10, 5 μg/IM	7	6 per dose level
13-15	Rec-Spike - MF59	20, 10, 5 μg/SC	7, 21, 35, 42 d	6 per dose level
16-18	Rec-Spike - MF59	20, 10, 5 μg/SC	7	6 per dose level
19-21	Rec-Spike - MF59	20, 10, 5 μg/IM	7, 21, 35, 42 d	6 per dose level
22-24	Rec-Spike - MF59	20, 10, 5 μg/IM	7	6 per dose level
25	MF59	NA/SC	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
27	MF59	NA/IM	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
29	Saline	NA/SC	7, 21, 35, 42 d	6 + 6 (sac d 7 and 42)
31	Saline	NA/IM	7, 21, 35,42 d	6 + 6 (sac d 7 and 42)

This protocol can also be used to assess the Th1/Th2 profile of the specific immune response elicited by the recombinant Spike protein. Neutralizing and Spike-specific antibody titers will be assessed at days 7, 21, and 35; IgG2a *vs* IgG1 isotype of the Spike-specific antibodies will be determined at days 21 and 35; *in vitro* proliferation of lymph node and splenic T cells against the recombinant Spike protein will be determined at days 7 and 42, respectively; IFN-γ and IL-4 production by splenic T cell against the recombinant Spike protein from SARS-CoV will be assessed at day 42. Peripheral blood will be collected at days 7, 21, 35; lymph nodes cells at day 7, and spleen cells at day 42. Neutralizing and Spike-specific antibody titers and isotypes will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Proliferation of lymph node and splenic cells will be determined by ^{3}[H]–Thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing T lymphocytes, will be determined by ELISPOT and FACS.

EXAMPLE 8 -Immunogenicity, dosing and route schedule for Spike proteins in rabbits

The immunogenicity, route and dosing of the recombinant spike proteins of the invention in rabbits may be assessed using the below detailed protocol. Increasing doses can be tested in the range from 5 to 40 μg of recombinant Spike antigen alone or mixed with an equal volume of MF59-citrate, administered SC or IM to anesthetized animals in 200µl of inoculum. Groups of New Zealand white female rabbits, 10 per treatment, will be immunized as shown in the table below. The animals will be primed at day 0 and boosted at days 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibody titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively.

Group	Treatment	Dose/Route	Sampling interval	Number of rabbits
1-4	Full-length Spike protein	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
5-8	Full-length Spike protein	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
9-12	Truncated Spike protein	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
13-16	Truncated Spike protein	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
17-20	Full-length Spike protein - MF59	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
21-24	Full-length Spike protein - MF59	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
25-28	Truncated Spike protein - MF59	40, 20, 10, 5μg/SC	7, 21, 35 d	10 per dose level
29-32	Truncated Spike protein - MF59	40, 20, 10, 5μg/IM	7, 21, 35 d	10 per dose level
33	MF59	NA/SC	7, 21, 35 d	10
34	MF59	NA/IM	7, 21, 35 d	10
35	Saline	NA/SC	7, 21, 35 d	10
36	Saline	NA/IM	7, 21, 35 d	10

EXAMPLE 9 - Immunogenicity and dose schedule for recombinant Spike in ferrets

The immunogenicity and dosing of the recombinant spike proteins of the invention in ferrets may be assessed using the below detailed protocol. Three groups of ferrets, 6 for treatment, will be immunized with recombinant SARS-CoV Spike protein from CHO cell lines, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 200μl of inoculum. The recombinant Spike protein vaccine will be tested at the dose eliciting the highest neutralizing antibody titers in mice at day 35 after the second boost. The animals will be primed at day 0 and boosted at day 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively.

Groups	Treatment	Dose/Route	Sampling interval	Number of ferrets
1 & 2	Rec-Spike protein	Y μg or 2Y μg /SC	7, 21, 35 d	6
3 & 4	Rec-Spike protein + MF59	Y μg or 2Y μg/SC	7, 21, 35 d	6
5	Saline	NA/SC	7, 21, 35 d	6

The 3 groups of ferrets, 6 animals per group, used for the immunogenicity studies above can then be used to assess efficacy of the recombinant Spike protein in protecting vaccinated animals from infection and/or disease. Anestethized animals will be challenged two weks after the last boost intratracheally with 10^6 median tissue culture infectious dose unit ($TCID_{50}$) of the SARS-CoV Utah strain. Infection by SARS-CoV will be assessed by taking nasal, faringeal and rectal swabs from animals for 20 days after challenge as described (12). The presence of SARS-CoV in sample materials will be assessed by RT-PCR and infection assay of Vero cells. Animals will be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection will be determined by the magnitude and duration of virus shedding and by duration and severity of disease symptoms and percentages of surviving animals.

EXAMPLE 10: Expression of Spike protein for vaccination

The SARS-CoV Spike glycoprotein was expressed in both full-length and truncated forms, using the nSh and nShΔTC pCMVIII constructs described above, both with hexahistidine tags. The vector constructs were evaluated for expression 48 hr after transfection into 293 cells and COS7 cells. The full-length Spike protein (nSh) was detected by western blot only in cell lysate, but not in culture media (Figure 52).

The majority of SARS-CoV full-length Spike protein was expressed in transiently-transfected COS7 cells as a high molecular glycoprotein which ran at 540 kDa in non-reducing gels (Figure 53). The gp540 is heat labile as indicated by the complete dissociation into monomeric forms (gp170 & gp180) by boiling, but it was resistant to DTT treatment. These data suggest that the recombinant Spike protein is noncovalently associated into a homotrimer (gp540). The presence of Spike protein in homotrimeric association also was confirmed in inactivated, purified SARS-CoV virion particles. Analysis of virion proteins by western blot under the same condition used for the characterization of recombinant Spike protein generated essentially identical results (Figure 54).

EXAMPLE 11: Spike protein processing

In order to characterize Spike protein processing, BHK-21 cells were infected with alphavirus replicon particles expressing the SARS-CoV full-length Spike. At 6 hoursr post-infection with an MOI of 5, infected cells were labeled for 1 hr with L-[^{35}S]methionine/cysteine and chased for up to 4 hours. The [^{35}S]-labeled spike protein was immunoprecipitated by anti-SARS rabbit serum and digested with Endo-H. Both digested and undigested proteins were analyzed by SDS-PAGE (4% polyacrylamide). As shown in Figure 55, the full-length spike protein is synthesized as an Endo-H sensitive high-mannose glycoprotein (gp170, an ER form) that undergoes modification to an Endo-H resistant glycoprotein with complex oligosaccharides (gp180, a Golgi form). The conversion of gp170 into the gp180 form takes place within 2 hours (Figure 56).

EXAMPLE 12: High-level protein expression

To develop a system for rapid expression of protein antigens, DNA transfection of 293 (human embryonic kidney) cells was used, to obtain milligram quantities of recombinant antigen. The most common method for culturing and transfecting 293 cells is in static or monolayer cultures. These procedures were modified by performing large-scale transfection of 293 cells in suspension and expanding the transfected cells in suspension culture for production of secreted or intracellular proteins. Several initial experiments were performed at the 100-milliliter scale cultures to determine optimum conditions, such as number of cells, type of

transfecting reagent (FuGENE 6, Lipitoid or RO-1538) and the ratio of DNA to transfection reagent. Based upon pilot experiments, FuGENE 6 was the best transfecting reagent.

The kinetics of gene expression was compared to other viral envelope glycoproteins, and the data suggest that stable protein expression peaks around 72 to 96 hours post-transfection, depending upon the gene of interest, and then significantly decreases thereafter. Thus, using the optimum conditions, the transfection process was scaled from 100 ml to 4 liters. The 4 liter culture can be used for rapidly producing 2-10 milligrams of protein antigens. To facilitate antigen purification and also maximize the yield and recovery of the purified protein, transfection conditions were optimized by using serum-free medium.

Bulk transfection procedure has been used for the expression of truncated and full-length Spike antigens. The kinetics of expression for truncated form of the spike protein is presented in Figure 56A. Expression of the truncated form of Spike protein peaked around 48 hrs and was stable until 72 hrs, therefore the cultures were harvested at 72 hrs post transfection.

Collected media were concentrated 20X and used for purification of truncated Spike protein by a very simple purification strategy where the truncated form of the spike was captured on GNA lectin followed by DEAE and ceramic hydroxyapatite column chromatography. The purified protein was analyzed on SDS-PAGE by silver stain (Figure 56B) and also by western blot (Figure 56C). Early efforts were able to purify the truncated form of the spike protein with >95% purity and approximately 50% recovery. The molecular mass of the truncated form of the Spike protein is approximately 170-180 kDa.

Full-length Spike protein was expressed in 293 cells using the bulk transfection strategy. The expression data suggest that, like the truncated form, expression peaked around 48 hrs post-transfection and remained stable until 72 hrs. However, contrary to the truncated form and as expected, full-length protein is not secreted, but rather is retained within the cells, as shown by the absence of any signal in western blots of cell culture supernatants. The full-length form of the protein was purified from Triton X-100 detergent-extracted cells. Full-length Spike protein was then captured on GNA lectin, followed by hydroxyapatite and SP chromatography. The calculated molecular mass of full-length spike protein is approximately 600 kDa, which is close to the theoretical mass for the trimer.

EXAMPLE 13: SARS virus seed cultures

A SARS-CoV reference seed virus propagated only in certified Vero cells will be used for the generation of the Master and Working Virus Seeds under GMP. A clinical specimen from the respiratory tract of a patient infected by the SARS-CoV is inoculated onto documented VERO cells, with certified culture media. Culture media containing the virus are harvested at 4 days post-infection and designated Passage 1 (P1). A second round of virus propagation is again

performed in certified VERO cells with certified media, by inoculation of 1 ml per T-75 flask of 100 times diluted P1 virus. Culture supernatant was harvested at 3 days post-infection and stored at -80°C as a P2 reference stock virus, without plaque purification.

Cell banks of Vero cells for further production of SARS-CoV are prepared from specific cell subsets that have not been used since the emergence of transmissible spongiform enephalopathies (*e.g.* since 1980). A research cell bank of these cells has been prepared using specified New Zealand-origin fetal bovine serum. From this research cell bank, a Master Cell Bank (MCB) is made under GMP conditions and using only specified and well-controlled media and supplements. The cell bank will is tested for absence of adventitious agents according to applicable US, EU, and international guidelines (see Points To Consider "Characterization of cell lines used to produce biologicals", FDA/CBER 7/1993; ICH Q5D Draft 6 "Cell substrates", Oct.23, 1996; CPMP/ICH/294/95 "Note for Guidance on Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (Step 4, 16. July 97); WHO final draft "Requirements for use of animal cells as in vitro substrates for the production of biologicals" 7.3.1997). Tumorigenicity and identity testing is also required for this cell bank.

The reference virus is plaque-purified and expanded in certified Vero cells in the absence of FCS in order to generate Master and Working Seeds. Another option to help ensure purity and facilitate the assessment of safety of the Master Seed is to subject the SARS-CoV to pelleting and resuspension in PBS. The virus suspension is made up to 60% (w/w) sucrose with crystalline sucrose, transferred to a centrifuge tube and overlayed with 50, 40, 30, and 20% (w/w) sucrose solutions in PBS. The gradient is centrifuged for 72h and then fractionated. The virus-containing fraction is diluted and the virions re-pelleted by ultracentrifugation. RNA from the virus pellet is isolated and transfected into certified Vero cells whereby the "infectious" positive-strand RNA will lead to the production of infectious virus, which can be plaque-purified and expanded to generate alternative Master and Working Seeds from purified virus RNA.

Viral seeds are tested for the absence of adventitious agents (see e.g. 21 CFR Revised as of April 1, 1994, § 630.35 Test for safety) and for identity, using a highly-specific neutralizing antiserum prepared from an independent source. Safety testing of viral seeds for vaccine purposes is done routinely by service laboratories. Broad-spectrum PCR testing can be used as an addition and/or alternative for testing.

EXAMPLE 14: Scale-up of virus production and inactivation

A protocol for the production, inactivation, and purification of inactivated SARS-CoV with sufficient structural integrity to elicit protective neutralizing antibody responses in animal models involves: Vero cells are infected with virus at an M.O.I. of 0.01 in the absence of FCS

and antibiotics; culture medium is collected, cleared by centrifugation, and inactivated with BPL, followed by confirmatory testing for complete inactivation; the inactivated material is filtered, subjected to MCS-column purification, and further purified by sucrose gradient centrifugation.

Several modifications and improvements can be developed when adapting this basic protocol to a larger scale for commercial use. Firstly, the cell culture and infection process can be adapted to roller bottles, as an intermediate step to allow rapid production for preliminary trials within existing BSL 3+ facilities. Full commercial production will typically use a fermentation process in a closed system, but a roller bottle system can be achieved more rapidly. The roller bottles do offer a true suspension culture system for Vero cells, which gives various technical and safety advantages over microcarrier cultures. Suspension cultures can be grown to any desired fermentation scale without interfering with the closed system between cell passages, as no trypsinization is required.

To scale up the infection process in roller bottles to 30-50 liters per batch, the optimum M.O.I. and harvesting periods for selected media and culture conditions should first be determined. For the larger scale, methods for harvesting and handling larger volumes of highly infectious material safely should be used, and so cell separation via centrifugation should be replaced by a method such as filtration through single-use filter cartridges.

The MCS-chromatography and the gradient purification steps described above can readily be scaled to a batch volume of up to 50 liters. For larger volumes, however, and for increased purity, ultrafiltration and sterile filtration steps will be used. Nuclease treatment to remove host cell DNA will also be included.

EXAMPLE 15: Large scale analytical methods

Analytical methods for the SARS coronavirus include virus titration methods, immunological and physico-chemical methods to quantitate and characterize the purified antigen (ELISA, PAGE, western blots using specific antisera against purified whole virus, *etc.*). Other analytical tests include: fast yield testing via asymmetric field flow separation and laser particle detection and counting; Western blot using specific antisera against individual viral proteins; and tests for residual host cell DNA.

Residual DNA testing is generally done by hybridization *e.g.* using a limit test. Such testing is performed according to methods already established and validated for other cell lines. As an alternative, the Threshold™ method may be used.

For producing specific antibodies, recombinant protein expression of all the ORFs from the structural and non-structural gene regions of the SARS-CoV is used. The ORFs can be cloned and expressed in *E.coli* and, if necessary, also in eukaryotic vectors such as baculovirus. This can provide sufficient amounts of purified soluble protein to immunize mice and rabbits to produce

polyclonal and monoclonal antibodies against SARS proteins and to set up specific ELISA assays. Different expression vectors can be tested to maximize the yield of recombinant protein in a soluble form *e.g.* different vectors, one containing sequences coding for six N-terminal histidine residues and another containing a Glutathione-S-transferase protein fused to the C-terminus of the SARS protein. The recombinant proteins can be purified by single step column chromatography on either Nickel chelating Sepharose or Glutathione-Sepharose 4B resin. These procedures are very rapid and generally produce protein of 60-90% purity, which is suitable for raising specific antisera (Pizza *et al.* (2000) *Science* 287:1816-20). Five mice and two rabbits for each recombinant protein can be immunized SC with 20 and 50 μg recombinant protein, respectively, given in IFA as adjuvant, at day 0, 14 and 28. Sera are collected at day 7, 21 and 35 to assess specific titers before euthanasia of the animals for collection of blood and removal of spleens.

For the detection of impurities (*e.g.* Vero cell derived proteins) in the vaccine preparation, rabbit serum reactive against Vero-derived proteins can be used. Such antisera are obtained by immunizing rabbits with at least 10μg of Vero cell lysate with CFA/IFA. The sera can be verified for reactivity against Vero-derived proteins in western blots. For more specific antisera against specific relevant cell-derived proteins that tend to be co-purified with the virus, mock-infected cell culture harvest that have undergone the purification process can be prepared and used for immunizing rabbits.

Methods to determine neutralization titers of sera from immunized animals and humans can be developed, without the constraints of using infectious SARS-CoV in a BSL-3+ laboratory. One such strategy will be to use recombinant antigens, particularly Spike protein or Spike-derived epitopes, and to develop ELISA assays for measuring antibodies against the target protein. Suitable epitopes allow a correlation to be established between the ELISA values and virus neutralization assay values. This approach provides a faster and more efficient (higher-throughput) comparison of specific and protective antibody titers. This ELISA test is also the ideal tool to monitor specific antibodies in safety trials, where several hundred animal sera must be tested.

Another strategy is to combine structural elements from both the pathogenic SARS-CoV and the non-pathogenic coronavirus mouse hepatitis virus (MHV) to construct chimeric virus-like particles (VLPs) that can be labeled. The assay is based on fusion between octadecyl rhodamine (R18)-labeled VLPs and cells (Hoekstra *et al.* (1984) *Biochemistry* 23:5675-81). The method relies on the relief of fluorescence self-quenching of R18 incorporated into VLPs upon fusion with cellular membranes. Coronavirus VLPs have been shown to mimic native virions with respect to their appearance in the electron microscope (EM) and their biological activities. As they do not contain viral RNA, however, then they cannot cause a productive infection

(Vennema *et al.* (1996) *EMBO J* 15:2020-2028). The VLP system can be used for the mouse hepatitis virus (MHV) strain A59 (MHV-A59))Godeke *et al.* (2000) *J Virol* 74:1566-15) containing a chimeric S protein. The protein chimera, consisting of the ectodomain of the SARS-CoV and the transmembrane and endodomain (64 C-terminal amino acid residues) from the MHV spike protein, can be co-expressed with the MHV M (membrane) and E (envelope) protein in OST-7 cells)Godeke *et al.*). VLPs secreted in the supernatant are harvested, purified and labeled with octadecyl rhodamine (R18) (Hoekstra *et al*). A constant amount of VLPs is incubated with a serial dilution of sera at 37°C for 1 hour in a 96-well plate. Subsequently, cells expressing the receptor for the SARS-CoV, the angiotensin-converting enzyme 2 (ACE2) (Li *et al.* (2003) *Nature* 426:450-54) is be added and the extent of fusion can be measured with a fluorescence spectrophotometer.

A final strategy to monitor the ability of sera to inhibit cell-cell fusion interactions between cells expressing the SARS-CoV S protein and a human cell line expressing the angiotensin-converting enzyme 2 (ACE2), a functional receptor for SARS-CoV (Li *et al.*). This reporter gene-based assay uses the fluorescent shift (green to blue) of the fluorogenic substrate CCF2/AM (AM=acetoxymethyl) upon cleavage by β-lactamase (Bla) as read-out for cell-cell fusion (Zlokarnik *et al.* (1998) *Science* 279:84-88). For this assay, a BHK-derived cell line, stably expressing Bla and the SARS-CoV S protein is generated. In addition, a human cell line expressing ACE2 on its surface is used. BHK cells, expressing the S protein on their surface and Bla in their cytosol are incubated with serial dilutions of the sera to be tested for 1h at 37°C. The cell line expressing the ACE2 is loaded with 1μM CCF2/AM for 1 h at 22°C, washed twice with PBS, and co-cultivated with the BHK cells. In case of cell-cell fusion, Bla cleaves the substrate, resulting in a green blue shift with excitation at 409 nm. Inhibition of fusion by sera thus provides a detectable change.

EXAMPLE 16: Stabilisation of inactivated SARS-CoV

Although the purified inactivated SARS-CoV vaccine is capable of inducing potent neutralizing antibody responses in animals, it is relatively instable and can benefit from formulation to increase stability for an acceptable period of time. Suitable formulation changes include the use of various buffer systems, pH ranges, stabilizing excipients (*e.g.* sugars and sugar alcohols, amino acids, *etc.*) *etc.*. Stability testing can be conducted in real-time at normal storage temperatures, or can be conducted in an accelerated manner by using elevated temperatures. Vaccine stability can thus be increased to approximately one year or longer. Lyophilized vaccine formulation can also be used to extend shelf-life, possibly with further additives for stability during lyophilisation.

EXAMPLE 17: Dose and schedule optimization for inactivated virus

Animal models of SARS-CoV infection have been reported, including mice, ferrets and macaques. As mentioned in example 4 above, mice immunized with the BPL-SARS-CoV vaccine achieve neutralizing antibody titers in the range of 1:100 – 1:1000, similar to levels found in convalescent patients, and are 100% protected from infection with a challenge virus. While the mouse challenge model is limited only to infection but not disease, ferrets and macaques are useful models of the human SARS disease. Two to four days after inoculation with SARS-CoV, both ferrets and macaques have been found to shed infectious SARS-CoV particles from the throat, nose and pharynx, as demonstrated by RT-PCR and/or virus isolation on Vero cells. At approximately the same time, the infected animals became lethargic, show respiratory distress and eventually die. Histologically, SARS-CoV infection in these animals associates with pulmonary lesions of different severity, similar to those found in biopsied lung tissue and autopsy material from SARS patients. With the availability of these models, preclinical studies with vaccines can be performed initially in mice for immunogenicity readouts, while efficacy of optimal doses and schedules can be assessed in the ferret and macaque models.

Initial studies in mice are used to determine the optimal dose and schedule required to elicit the highest levels of neutralizing antibody, with titers at least in the range of 1/100 – 1/1000. In parallel to the assessment of neutralizing activity, other features of the humoral immune response and cellular immune responses can be investigated. In particular sera from immunized mice can be assessed for the isotype (IgG1 *vs.* IgG2a) of the Spike-specific antibody response. Also, the frequencies of splenic CD4+ T cells producing IFN-γ and IL-4 in response to BPL-SARS-CoV particles will be assessed by ELISPOT and ELISA. These experiments can provide insight into the quality of the T cell response helping the priming of a protective antibody response.

Increasing vaccine doses can be tested (*e.g.* from 5 to 20 μg of BPL-SARS-CoV alone or mixed with an equal volume of MF59-citrate), administered SC to anesthetized mice in 100μl of inoculum. Groups of BALB/c mice, 10 per treatment, are immunized, with priming at day 0 and boosting at days 14 and 28. Secondary endpoints compare the kinetics of neutralizing *vs.* Spike-specific antibody titers and assess the Th1/Th2 profile of the specific immune response, and so neutralizing and Spike-specific antibody titers are assessed at days 7, 21, 35, and at 2, 3, 4, and 5 months after priming. The IgG2a and IgG1 titers of Spike-specific antibodies are determined at days 21, 35, and at 2, 3, 4, and 5 months after priming. Proliferation and IFN-γ and IL-4 production by splenic T cells against recombinant Spike protein from SARS-CoV are assessed at day 42, and at the end of the 5th month. Peripheral blood is collected at days 7, 21, 35, and at 2, 3, 4, and 5 months after priming. Spleen cells will be obtained at day 42 and at the end of the 5th month. Neutralizing and Spike-specific antibody titers and isotypes are determined by inhibition

of infection of Vero cells and by ELISA, respectively. Proliferation of splenic cells is determined by ^{3}[H]-thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing CD4^{+} T lymphocytes is determined by ELISPOT and FACS analysis.

Based on mouse results, the BPL-SARS-CoV vaccine can be tested in ferrets for the induction of protective neutralizing antibody titers. Ferrets are immunized according to a similar schedule as the mice and at the dose that elicits the highest neutralizing antibody titers in mice at day 35 after the second boost. Three groups of ferrets, 6 per treatment, are immunized with BPL-SARS-CoV, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 200μl of inoculum. The animals are primed at day 0 and boosted at days 14 and 28. Peripheral blood is collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers are determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Each group of ferrets is used to assess efficacy of the BPL-SARS-CoV in protecting vaccinated animals from infection and/or disease. Anesthetized animals are challenged intratracheally, two weeks after the last boost, with 10^{6} median tissue culture infectious dose units ($TCID_{50}$) of the SARS-CoV CDC strain. Infection by SARS-CoV can be assessed by taking nasal, pharyngeal and rectal swabs from animals for 20 days after challenge (Martina *et al. supra*). The presence of SARS-CoV in sample materials can be assessed by RT-PCR and infection assay of Vero cells. Animals can be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection can be determined by the magnitude and duration of virus shedding, by duration and severity of disease symptoms, and by percentage of surviving animals. The formulation eliciting the highest neutralizing antibody titers at day 35 can then be tested against a two-fold higher dose of BPL-SARS-CoV given in the same formulation in the same regimen.

Additional studies can evaluate immunogenicity and efficacy of the candidate vaccine in non-human primates. Three groups of adult cynomolgus macaques, 4 per treatment, are immunized with BPL-SARS-CoV, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 500μl of inoculum. The BPL-SARS-CoV vaccine can be tested at the dose eliciting the highest neutralizing antibody titers in ferrets at day 35 after the second boost. The animals are primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood is collected at weeks 1, 4, and 7. A secondary endpoint is to assess the Th1/Th2 profile of the specific immune response. Neutralizing and Spike-specific antibody titers and frequencies of peripheral blood CD4+ T cells producing IFN-γ and IL-4 in response to the recombinant SARS-CoV Spike protein is thus assessed at weeks 1, 4, and 7. Neutralizing and Spike-specific antibody titers can be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Intracellular cytokine staining and FACS analysis will be used to quantify IFN-γ- and IL-4-producing CD4^{+} T cells. The macaques can also be used to assess efficacy of

the BPL-SARS-CoV in protecting vaccinated animals from infection and/or disease. Anesthetized macaques can be challenged two weeks after the last boost with 10^6 median tissue culture infectious dose unit ($TCID_{50}$) of the SARS-CoV CDC strain in a 5 ml volume. A few drops of the virus can also be administered on each of the conjunctiva, 0.5 ml in the nose and the remainder in the trachea. Infection by SARS-CoV can be assessed by taking nasal, pharyngeal, and rectal swabs, and feces from animals for 20 days after challenge (Fouchier *et al.* (20030 *Nature* 423:240). The presence of SARS-CoV in sample materials can be assessed by RT-PCR and infection assay of Vero cells. Animals can also be monitored for clinical signs of SARS disease by assessing sleeping time, temperature, respiratory symptoms, diarrhea, body weight and survival. Protection can be determined by the magnitude and duration of virus shedding, by duration and severity of disease symptoms, and by percentage of surviving animals.

Mice

Group	Treatment	Dose/Route	Sampling interval	Number of mice
1-3	BPL-SARS-CoV	20, 10, 5 μg/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 per dose level
4-6	BPL-SARS-CoV	20, 10, 5 μg/SC	42 d	10 per dose level
7-9	BPL-SARS-CoV MF59	20, 10, 5 μg/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 per dose level
10-12	BPL-SARS-CoV MF59	20, 10, 5 μg/SC	42 d	10 per dose level
13	MF59	NA/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 + 10 (sacrificed at 42 d and end 5 m)
14	Saline	NA/SC	7, 21, 35 d; 2, 3, 4, 5 m;	10 + 10 (sacrificed at 42 d and end 5 m)

Ferrets

Group	Treatment	Route	Sampling interval	No. of ferrets
1	BPL-SARS-CoV	SC	7, 21, 35 d	6
2	BPL-SARS-CoV-MF59	SC	7, 21, 35 d	6
3	Saline	SC	7, 21, 35 d	6

Macaques

Group	Treatment	Route	Sampling interval	No. of macaques
1	BPL-SARS-CoV	SC	1,4, 7 w	4
2	BPL-SARS-CoV – MF59	SC	1,4, 7 w	4
3	Saline	SC	1,4, 7 w	4

EXAMPLE 18: Human T cell responses

As a prelude to initiation of clinical studies in humans, the reactivity of peripheral blood T lymphocytes from healthy donors with different HLA haplotypes can be assessed using the *in vitro* priming technique (Abrignani *et al.* (1990) *Proc Natl Acad Sci U S A* 87:6136-40). The aim of this study is to have a first indication of the immune-dominant T cell epitopes in SARS-CoV

proteins. Briefly PBMCs from 20 healthy donors with different HLA haplotypes will be cultured in medium containing 5% autologous serum, in the presence of different concentration of SARS-BPL-CoV particles in the range from 0.5 to 20 μg/ml. The expression of activation markers will be assessed after 24 and 48 hours. Frequencies of IFN-γ- and IL-4- producing T lymphocytes will be assessed after 12h and after 15 days in culture, in the presence of 100 U/ml recombinant human IL-2. Activated and cytokines producing CD4 T lymphocytes will be sorted and eventually cloned as single cells using FACS technologies. The CD4+ T cell repertoire from human subjects with different HLA will be assessed by proliferation assays of the CD4+ T cell lines and clones against autologous EBV-transformed cell lines loaded with 15-mer overlapping peptides from the most relevant structural and non structural protein of the SARS-CoV.

When moving to actual human trials, safety and immune responses will be evaluated in healthy adults following intramuscular immunization with escalating doses of the BPL-inactivated SARS-CoV vaccine, with MF59 adjuvant being included or omitted depending on preclinical data. Three/four immunizations will be given at 0, 1, 6 months in the first cohort, and at 0, 1, 2, 6 months and 0, 2, 6 weeks in the second and third cohorts respectively. The trial will be observer blind and placebo controlled. Subjects will be randomized into each dose level. Immune response parameters to be measured will include serum neutralizing antibodies, ELISA antibodies and peripheral blood IFN-gamma-producing CD4+ T cells by intracellular cytokine staining.

Group	Antigen dose (μg)	Administration schedule	No. treated subjects	No. subjects with placebo	Sampling interval
A1	10	0,1,6 months	18	6	0, 1, 2, 6, 7 mos
A2	20	0,1,6 months	18	6	0, 1, 2, 6, 7 mos
B1	10	0,1,2,6 months	18	12	0, 1, 2, 6, 7 mos
B2	20	0,1,2,6 months	18	12	0, 1, 2, 6, 7 mos
C1	10	0,2,6 weeks	18	12	0, 2, 6, 10, 30 wks
C2	20	0,2,6 weeks	18	12	0, 2, 6, 10, 30 wks

EXAMPLE 19: Selection of CHO cell lines for Spike protein expression

Methods for the derivation of Chinese Hamster Ovary (CHO) cell lines that stably express viral envelope glycoproteins that are conformationally intact, appropriately glycosylated and efficiently bind neutralizing antibodies are well established for HIV and HCV (Srivastava *et al.* (2002) *J Virol* 76:2835-47; Srivastava *et al.* (2003) *J Virol* 77:11244-259; Heile *et al.* (2000) *J Virol* 74:6885-92). The same techniques can be applied to SARS-CoV, to generate two different stable CHOK-1 cell lines producing either full-length or truncated SARS Spike proteins. The Spike proteins can be expressed using the constructs described herein, but without the hexa-His tags. These proteins can compared for their ability to produce neutralizing antibodies in immunized animals as well as for their expression levels in CHOK-1 cells.

A pCMV3 vector expressing Spike can be used for the derivation of stable CHOK-1 cell lines, containing the CMV enhancer/promoter, ampicillin resistance, and a fused DHFR and attenuated neomycin gene for selection purposes. Stable cell lines can produced using the neomycin selection system in CHOK-1 cells. Clones can be sequenced to verify the integrity of the insert, and transient transfections can be performed using Trans-LT1 polyamine transfection reagent (PanVera Corp., Madison, WI) to assess the expression level and also the integrity of the expressed protein by ELISA and western blot analysis.

Initial CHO cells will be selected to be free from TSE/BSE contaminants and risks according to relevant regulatory standards. To construct cell lines, procedures involve transfection, primary screening with selective medium, followed by subcloning to assure purity of cell lines. Cell supernatants can be assayed using an antigen capture ELISA to quantify expression levels at all stages of selection and amplification. For full-length Spike expression, methanol fixed cells can be screened for internal expression by immunofluorescent staining using a rabbit anti-SARS antibody. Successive measurements at the T75-flask stage of expansion canbe employed to assure stability of expression levels. The molecular mass and integrity of the expressed proteins can be checked by PAGE both under native and reducing and denaturing conditions, followed by immunoprobing.

The pCMV3 vectors expressing SARS-CoV Spike proteins in either full-length or truncated forms can be introduced into CHOK-1 cells using the Trans-LT-1 reagent and non-selective media. 24-48 hours post-transfection, depending on cell density, cells are split at a 1:5 ratio and the medium can be changed to selective media containing neomycin at 500μg/ml. Any bovine serum used in these procedures will be from TSE-free sources that meet regulatory standards. Ten to fourteen days later, individual colonies can be picked and transferred to 96 well plates and cultured in complete non-selective medium. When approximately 80% of the wells are confluent, 24 hour supernatants can be screened by Spike capture ELISA. For initial expression of full length Spike protein, cells can be fixed with methanol and screened by immunofluorescent staining using a rabbit anti-SARS antibody. After low-expressing cell lines have been eliminated and there are fewer than 20-30 cell lines, capture ELISA and western blots can then be used to determine the expression level after cell lysis. A portion of each cell line can be pelleted, weighed and lysed in 1% Triton lysis buffer for determination of expression levels. Three to four clones producing the highest levels of spike protein in correct structure and conformation can be expanded to three-liter bioreactors and adapted to low serum suspension culture conditions for scale-up.

The antigen capture ELISA assay for the SARS spike protein can be performed using 96 well flat-bottom plates coated with 250ng per well of purified immunoglobulin obtained from rabbit sera that were immunized with inactivated SARS virus. Supernatant or lysate samples are

added and incubated for 2 hours at 37°C. Bound antigen is reacted against pooled $SARS^{+ve}$ serum or high affinity monoclonal antibody either human or mouse against SARS spike protein and detected using appropriate species-specific peroxidase-conjugated second antibody. The plates are developed using TMB substrate (Pierce, Rockford, IL), read at a wavelength of 450nm, and the concentration of protein per ml sample is derived from a standard curve (OD *vs.* protein concentration) based on serial dilutions of a known concentration of recombinant spike protein.

The immunoprobing analysis will also be performed following the standard methods described by Srivastava *et al.* (2002) *supra.* Briefly, 10-20μl of the sample is analyzed on 4-20% SDS PAGE under non-reducing/denaturing conditions with mild heating. The proteins are then transferred onto nitrocellulose membranes and reacted against polyclonal anti-Spike rabbit serum, followed by anti-rabbit Ig conjugated to Alexa 688 (Molecular Probes, Oregon). The blots are scanned using an infrared imaging system.

The highest expressing candidate cell lines will be screened for Spike protein expression and stability in small-scale (3 liter) perfusion bioreactors. The candidate clones will be further evaluated for level of expression as well as integrity of expressed protein, and subsequently tested for expression stability in the absence of selection. The selected clones also will be tested for maintenance of the DNA sequence integrity of the integrated SARS spike protein gene. To quickly monitor the expression levels in small flasks and in the three liter evaluation cultures, a lectin-based process (Gluvanthus Nivalis lectin) has been developed to isolate SARS spike protein to a degree of purity that allows semi-quantitation and characterization of the protein in CHO supernatant. Full-length Spike protein will be obtained from Triton X-100 detergent extracted cells and then captured on GNA lectin, followed by hydroxyapatite and SP chromatograph. Eluted protein is then characterized by: (1) polyacrylamide gel electrophoresis (PAGE) and Coomassie staining, (2) immunoprobing with anti-SARS rabbit sera, (3) structural characterization using size exclusion chromatography (SEC), as well as mass spec analysis using MALDI-TOF.

Productivity from the CHO cell line expressing SARS spike protein should be at least 2 mg/L and for full-length Spike protein will be 3mg/100gm of cells, at steady-state cell density. Yield from one 45 day, 2.5-liter bioreactor will be ~1000 mg crude protein.

EXAMPLE 20: Purification of spike protein for human vaccines

To purify SARS spike protein for the purpose of producing GMP grade material for human use, the following basic process is used, with all steps being performed at 2-8°C: the starting material, concentrated CHO cell culture supernatant (20-30X) is thawed and filtered through a 0.45μm membrane; this material is heavily contaminated proteins from culture, as well as DNA;

the first purification step is affinity chromatography using Gluvanthus Nivalis (GNA), a lectin that preferentially recognizes terminal mannose containing carbohydrates; glycosylated proteins, including SARS spike protein are captured and non-glycosylated proteins, as well as DNA, do not bind to this column; the GNA column is followed by two chromatographic steps operated in the flow through mode; the anion exchanger, DEAE, and ceramic hydroxyapatite (cHAP); DEAE binds some contaminating supernatant proteins and DNA, whereas cHAP binds any contaminating serum proteins; full-length Spike protein is purified from the cell pellet; the cells are lysed with Triton X-100 and full-length Spike protein is then captured on GNA lectin, followed by hydroxyapatite and SP chromatography.

The purified SARS spike can be further treated to remove adventitious viruses: viral inactivation at pH 3.5 for 1 hour; the sample is then concentrated and diafiltered into a buffer at pH 4 and finally captured the purified protein using SP resin; the spike protein binds to this resin and many viruses flow through.

The spike protein is eluted, concentrated and diafiltered into formulation buffer. This formulated bulk product is then filtered through a DV50 viral removal membrane followed by filtration through a 0.2 μm membrane. The formulated bulk is filled into suitable containers *e.g.* into 3.0 ml vials, in a class 100 laminar flow hood.

In process testing at each step of the purification includes protein concentration, endotoxin (LAL), bioburden, and recovery.

Prior to human administration, a test for potency will evaluate the specific ability of the vaccine in an *in vitro* or *in vivo* test to effect a given response. The *in vivo* immunogenicity will be determined by dosing groups of 10 mice with various doses of the protein antigen. Sera will be analyzed for the presence of IgG antibodies using an ELISA. The criterion for passing will be based upon the number of vaccine treated animals that are seropositive compared to a reference standard. Other tests include General Safety, sterility, purity, identity of the vaccine (using an ELISA specific for Spike protein), and quantity & protein concentration (UV spectrophotometric absorbance procedure based on the molar absorbance of the aromatic amino acids).

Stability testing will be performed on the bulk drug substance and on the final container product. Bulk product will be evaluated at temperatures of –60°C (recommended storage condition), 25 ±2 °C and 40 ±2 °C protected from light, at time points of 0, 3, 6, 9, 12 months. Final container product will be tested at temperatures of –60 °C, and inverted at 5 ±3 °C, 25 ±2 °C, and 40 ±2 °C at time points of 0, 3, 6, 9, 12 months. Stability-indicating assays may include appearance, pH, protein content, SDS-PAGE, size exclusion HPLC, and container/closure integrity, performed on single samples of bulk and triplicate vials of final container material.

The protein purified in this way can be evaluated in mice, rabbits and ferrets as described in, and based on the results of, examples 4, 5, 8 and 9 above.

Initial experiments will be performed in mice to determine optimal dose and schedule of the GMP Spike protein required to elicit the highest levels of neutralizing antibody, with titers at least in the range of 1/100 – 1/1000. Spike protein will be tested in the range from 5 to 40 μg, alone or mixed with an equal volume of MF59-citrate, to anesthetized mice in 100μl of inoculum. Groups of BALB/c mice, 10 per treatment, will be immunized. The animals will be primed at day 0 and boosted at days 14 and 28. Secondary endpoints will be to compare the kinetics of neutralizing *vs.* Spike-specific antibody titers and to assess the Th1/Th2 profile of the specific immune response. Neutralizing and Spike-specific antibody titers will be assessed at days 7, 21, and 35 and at 2, 3, 4, and 5 months after priming; the IgG2a and IgG1 titers of Spike-specific antibodies will be determined at days 21 and 35, and at 2, 3, 4, and 5 months after priming; proliferation and IFN-γ and IL-4 production by splenic T cell against the recombinant Spike protein from SARS-CoV will be assessed at day 42 and at the end of the 5th month. Peripheral blood will be collected at days 7, 21, and 35 and at 2, 3, 4, and 5 months after priming; spleen cells at day 42 and at the end of the 5th month. Neutralizing and Spike-specific antibody titers and isotypes will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Proliferation of splenic cells will be determined by ^{3}[H]–thymidine uptake. Frequencies of splenic IFN-γ and IL-4 producing CD4+ T lymphocytes, will be determined by ELISPOT and FACS analysis.

Next, the optimal dosing and schedule for recombinant Spike vaccine will be determined in ferrets. Based on the mouse results, the Spike vaccine eliciting the highest antibody neutralizing titers will be tested against a two-fold higher dose of recombinant Spike protein given in the same formulation. Three groups of ferrets, 6 per treatment, will be immunized SC under anesthesia with 200μl of inoculum. The animals will be primed at day 0 and boosted at days 14 and 28. Peripheral blood will be collected at days 7, 21, and 35. Neutralizing and Spike-specific antibodies titers will be determined by inhibition of SARS-CoV infection of Vero cells and by ELISA, respectively. Similar to the previous ferret studies, each group of animals will be used to assess efficacy of the vaccine in protecting immunized animals from infection and/or disease.

Immunogenicity and efficacy of the candidate vaccine also will be evaluated in nonhuman primates. Three groups of adult cynomolgus macaques, 4 per treatment, will be immunized with recombinant SARS-CoV Spike protein, alone or mixed with an equal volume of MF59-citrate, administered SC to anesthetized animals in 500 μl of inoculum. The Spike protein vaccine will be tested at the dose eliciting the highest neutralizing antibody titers in ferrets at day 35. The animals will be primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood will be collected at weeks 1, 4, and 7. A secondary endpoint will be to assess the Th1/Th2 profile of the specific immune response, as described above (neutralizing and Spike-specific antibody titers,

frequencies of peripheral blood CD4+ T cells producing IFN-γ and IL-4 in response to the recombinant Spike protein, assessed at at weeks 1, 4, and 7).

Finally, human phase I, placebo-controlled, dose-escalation, safety/ immunogenicity trials will be performed for the IM recombinant SARS vaccine with MF59 adjuvant. The trial will evaluate safety and immune responses in healthy adults following immunization with escalating doses of SARS recombinant vaccine with MF59 adjuvant, administered intramuscularly. Three/four immunizations will be given at 0, 1, 6 months. The trial will be observer blind and placebo controlled. Subjects will be randomized into each dose level. Immune response parameters to be measured include serum neutralizing antibodies, ELISA antibodies and peripheral blood IFN-γ-producing CD4+ T cells by intracellular cytokine staining:

Group	Vaccine Antigen dose (μg)	Administration schedule	No. of treated subjects	No. of subjects with placebo (MF59)	Sampling interval
A1	50	0,1,6 months	18	6	0, 1, 2, 6, 7 months
A2	100	0,1,6 months	18	6	0, 1, 2, 6, 7 months

EXAMPLE 21: Comparison of inactivated virus and purified Spike protein

Immunogenicity and efficacy of the inactivated virus vaccine and the purified Spike protein can be compared in non-human primates. Three groups of adult cynomolgus macaques, 4 for treatment, will be immunized with recombinant SARS-CoV Spike protein from CHO cell lines or with BPL-SARS-COV, given in the dose and formulation eliciting the highest neutralizing antibody titers in previous immunogenicity challenge experiments, administered SC to anesthetized animals in 500μl of inoculum. The animals will be primed at day 0 and boosted at 3 and 6 weeks. Peripheral blood will be collected at weeks 1, 4, 7. A secondary endpoint will be to assess the Th1/Th2 profile of the specific immune response, as described above.

Group	Treatment	Dose/Route	Sampling interval	No. of macaques
1	Rec-Spike protein + or - MF59	Y μg /SC	1,4, 7 w	4
2	BPL-SARS-CoV + or - MF59	Y μg/SC	1,4, 7 w	4
3	Saline	NA/SC	1,4, 7 w	4

EXAMPLE 22: Expression in yeast

Yeast is a useful and inexpensive eukaryotic expression system. Yeast-expressed proteins are used in recombinant hepatitis B virus vaccines, and recombinant SARS antigens may also be expressed in yeast for vaccine purposes. Yeast-expression is also convenient for the production of antigens for preparing monoclonal and polyclonal antitobodies, or for use in serological assays.

The nucleocapsid protein (N) and two different versions of the spike glycoprotein (S) from SARS coronavirus FRA strain (AY310120) were cloned for expression in *S.cerevisiae:*

SARS N: aa 1 – 422 (coordinates 28120-29388 of AY310120 strain) – Fig.65

SARS spike: aa 14 – 1195 (transmembrane domain and cytoplasmic tail deleted) – Fig.66

SARS spike: aa 14 – 662 (S1 domain)

To make the S1 construct, a XhoI-NotI fragment of approximately 3733bp encoding the full-length spike glycoprotein was the starting point. PCR was used to amplify the full-length gene in two pieces: XbaI-BlnI of 2440bp and BlnI-SalI of 1306bp. These fragments were subcloned into commercial vectors (Novagen): pT7Blue2 XbaI-BlnI (5' end of spike glycoprotein) and pT7Blue2 BlnI-SalI (3' end of spike glycoprotein; Figure 58), respectively. The following primers were used in the subsequent PCR reactions: Spk-1 (5') SEQ ID NO: 9785; Spk-2 (5') SEQ ID NO: 9786; Spk-3 (5') SEQ ID NO: 9787; Spk-4 (5') SEQ ID NO: 9788.

E. coli HB101 competent cells were transformed with the PCR ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification and plasmid amplification of the desired subclones, it was desirable to eliminate the internal SalI site present in the XbaI-BlnI portion of the spike sequence in order to facilitate future cloning into the yeast expression vector (BamHI-SalI). Therefore, we prepared a CelII-MfeI vector from the pT7Blue2 XbaI-BlnI (5' end Spike) subclone to eliminated a 143bp sequence containing the SalI site. Kinased oligos DS1-6 (SEQ ID NOS: 9789-9794) were then ligated into the CelII-MfeI vector to replace the 143bp that were removed to mutate the SalI site (no aa changes), creating pT7Blue2.XbaI-BlnIΔsal.

The 5' XbaI-BlnI (from pT7Blue2.XbaI-BlnI ΔSal) and the 3' BlnI-SalI (from pT7Blue2 BlnI-SalI) spike glycoprotein inserts were gel-purified and ligated them into the p893-1 XbaI-SalI vector (a vector derived from pLitmus 38 (New England Biolabs) with the alpha-factor leader sequence cloned into the BamHI -SalI sites of the MCS). The resulting full-length SARS Spike coding sequence was named p893-1.SARS Spike 1255 #9 (Figure 58).

E.coli HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification of the positive clones, pT7Blue2 Xba-Bln ΔSal was chosen for use as a template for PCR reactions to amplify the Spike S1 1967 bp Xba-Sal fragment. The fragment was then subcloned into the p893-1 Xba-Sal vector, sequence verified, and named it p893-1.Spike S1 #11 (Figure 59).

In order to clone into the *S.cerevisiae* expression vector, pBS24.1, the 5' end of the S1 sequence had to be modified from XbaI to HindIII to allow ligation with the 3' HindIII end of the ADH2/GAPDH BamHI-HindIII promoter fragment. From pT7Blue2 Xba-BlnΔSal (described above) an AgeI-SalI 1943bp fragment was gel-purified. This fragment was ligated along with a

synthetic pair of HindIII-AgeI 30bp kinased oligos (S1-1+S1-2 creating the necessary 5' HindIII site) into the pSP72 HindIII-SalI commercial subcloning vector (named pSP72.SARS Spike S1 #2; Figure 59). S1-1 had SEQ ID NO: 9795 and S1-2 has SEQ ID NO: 9796.

After sequence verification of the positive clone from miniscreen DNA analysis, the HindIII-SalI fragment was gel purified. The 1365 bp BamHI-HindIII ADH2/GAPDH promoter fragment was ligated along with the 1973 bp HindIII-SalI S1 fragment into the pBS24.1 BamHI-SalI vector creating the genetically engineered pd.SARS Spike S1 #2 expression plasmid (Figure 60).

S.cerevisiae strain AD3 was transformed with pd.SARS Spike S1 #2 and single transformants were checked for expression after depletion of glucose in the medium. The recombinant protein was expressed at high levels in yeast, as detected by Coomassie blue staining. In particular, yeast cells were transformed with the SARS S1 expression plasmid using the Invitrogen S.c. EasyComp™ Transformation Kit. Expression in shown in Figure 57.

To express Spike 1195 protein, which does not contain the trans-membrane (TM) region or cytoplasmic tail that are present in the full-length SARS construct, the following series of genetic manipulations was performed:

From pT7Blue2 BlnI-SalI #11 (described above) a BlnI-DraI 1056bp fragment was gel purified. This fragment was ligated with a synthetic pair of 68bp DraI-SalI kinased oligos (DRS1+2; SEQ ID NOS: 9797 & 9798) into a pT7Blue2 BlnI-SalI vector (Figure 61). *E.coli* HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence confirmation the clone was named pT7Blue2 BlnI-Sal Spike 1195 #7. The 1126bp BlnI-SalI fragment encoding the 3' end of the Spike 1195 was gel purified (Fig.61).

In order to generate the XbaI-SalI Spike 1195 fragment, the 3109bp XbaI-PciI fragment was isolated from the p893-1.SARS Spike 1255 #9 (described above) and a 457bp PciI-SalI fragment from pT7Blue2.SARS Spike 1195 #7 (described above). The two fragments were cloned into the p893-1 XbaI-SalI vector, creating the p893-1.SARS Spike 1195 #34 plasmid (Figure 62).

To clone SARS Spike 1195 into the pBS24.1 Saccharomyces cerevisisae expression vector, it was necessary to modify the 5' end of the SARS Spike 1195 from XbaI to HindIII, as done for the Spike S1 expression clone described above. To begin, the 2416bp AgeI-BlnI fragment was isolated from p893-1.SARS Spike 1195 #34. This fragment was ligated with the synthetic HindIII-AgeI 30bp oligos (described above to generate the S1 protein for expression in *S.cerevisiae*) into the pT7Blue2 HindIII-BlnI vector. E. coli HB101 competent cells were transformed with the oligo replacement ligation product and plated on Luria agar plates,

containing 100μg/ml ampicillin. The desired clones were identified using miniscreen DNA analysis. After sequence verification of the positive clone and plasmid amplification of pT7Blue2.SARS 1195 5' HindIII-BlnI #10 (Figure 63), we isolated a 402bp HindIII-NcoI fragment and the 2044bp NcoI-BlnI fragment (Figure 63). It was necessary for the HindIII-BlnI isolation to be done in two steps to avoid cloning issues related to the internal HindIII site located at nucleotide number 1319 of the spike 1195 protein.

To assemble the BamHI-SalI-expression cassette of Spike 1195 into the pBS24.1 vector *E.coli* HB101 competent cells were transformed with the the BamHI-HindIII (ADH2/GAPDH promoter), HindIII-NcoI 402bp fragment, NcoI-BlnI 2044bp and the BlnI-SalI 1126bp fragments into the pBS24.1 BamHI-SalI vector. The samples were plated on Luria agar plates, containing 100μg/ml ampicillin. The desired clone was identified using miniscreen DNA analysis, thus creating the genetically engineered pd.SARS Spike 1195 #10 (Figure 64).

S.cerevisiae strain AD3 was transformed with pd.SARS Spike 1195 #10 and single transformants were checked for expression after depletion of glucose in the medium. The recombinant protein was detected by Coomassie blue staining. In particular, yeast cells were transformed with the SARS 1195 expression plasmid using the Invitrogen S.c. EasyCompT™ Transformation Kit.

EXAMPLE 23: Expression in mammalian cell lines

cDNA fragments containing the S protein ORF of 1255 amino acids were amplified by RT-PCR from SARS viral RNA (Frankfurt isolate) grown in Vero cells. The amplified PCR fragments were cloned into pBlueScript vector, sequenced, and consensus spike sequence was assembled to create a full-length SARS spike clone, pBSnSh. *In vitro* transcription of pBSnSh followed by translation in a rabbit reticulocyte lysate resulted in the production of single polypeptide with an estimated molecular mass of ~140 kDa.

The insert of this plasmid was recloned via XhoI and Not I into a mammlian expression vector pCMVIII (Srivastava *et al.* (2003) *J. Virol.* 77:11244-11259) to create a construct, nSh (Fig. 74A). A PCR fragment containing a spike protein of 1195 amino acid, which was deleted for transmembrane (TM) domain and cystein-rich cytoplasmic tail (Cy) was amplified and cloned pCMVIII vector to generate the contstruct nShΔTC (Figure.74B). Both constructs were tagged with six histidine residues at the C-terminus in order to aid in their characterization. The Xho I/Not I fragment without a histidine tag also was subcloned into the alphavirus replicon vector backbone pVCRchim2.1 for use in the production of an alphavirus replicon particle chimera that expresses S protein. Production and characterization of the replication defective alphavirus vector particles was performed essentially as described previously (Perri *et al.* (2003)

J. Virol. 77:10394-10403; Polo *et al.* (1999) *PNAS USA*. 96:4598-4603). The resultant alphavirus vector particles were named as VEE/SIN.

COS7 cells and BHK-21 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C and 5% CO_2 in air. COS7 cells were transfected with expression plasmids (nSh, nShΔTC) using a transfection kit (TransIt-COS, Mirus) following the manufacturer's protocol. The cells were washed once with ice-cold PBS and lysed with 1x Lysis buffer (20mM MOPS, 10mM NaCl, 1.5mM $MgCl_2$, and 1% Triton X-100) containing complete mini protease inhibitor (Roche). After a 30-min incubation on ice, the debris was cleared by centrifugation. The cleared lysate was either purified or used directly in western blotting.

To purify secreted spike proteins, medium from transfected cells was collected and subjected to centrifugation at 12,000 rpm for 10 min to remove cellular debris. The cleared medium was applied to a ConA-agarose column (Vector Lab). The column was washed extensively with 20mM sodium phosphate buffer, and then the bound proteins were eluted with 1M methyl α-D-mannopyranoside (MMP), 1M NaCl in 20mM sodium phosphate buffer. Column fractions containing SARS-CoV spike proteins were applied to MagneHis Protein purification system (Promega) following the protocol suggested by the manufacturer.

For western blot analysis, proteins were separated by 4-20% SDS-PAGE and then transferred electrophoretically to nitrocellulose membrane (Invitrogen). Membrane was blocked in blocking buffer (5% skim milk and 0.1% Tween 20 in PBS) and incubated with indicated antibody at room temperature for 1 hr, washed and probed with horseradish peroxidase (HRP)-conjugated secondary antibody (Biosource) followed by chemiluminescence (ECL system, Amersham) and exposed by X-ray films. The antibodies used were a mouse monoclonal anti-histidine antibody (anti-His•tag Mab, Novagen), a rabbit polyclonlal antipeptide antibody against SARS-CoV spike proten (SmPab, Abgent), or rabbit anti-SARS sera (2BE) obtained by immunization of rabbits with purified SARS-CoV virion. The latter has a cell culture neutralizing titer of 1/2,500. Unless stated otherwise, antibody was used at 1/1,000 for anti-histidine antibody and SmPab and 1/10,000 for anti-SARS rabbit sera.

Some spike proteins were treated with Peptide-N glycosidase F (PNGase F). Cell lysates were diluted in 0.5% SDS and 1% β-mercaptoethanol and denatured at 100°C for 10 min. After 2-fold dilution with 1% NP-40 in 50mM sodium phosphate (pH 7.5), the samples were treated with PNGase F (NEB) at 37°C for 1 hr. Enzyme-treated samples were analyzed by 4-12% SDS-PAGE in reducing condition. For a partial digestion with the PNGase, the cell lysates were diluted with 50mM sodium phosphate (pH 6.0) containing 0.75% Triton-X and treated with PNGase F (Calbiochem) at 37°C for 3 hr. Enzyme-treated samples were analyzed by 4-20% SDS-PAGE in nonreducing condition.

Western blots of cells 48-hours after transfection are shown in Figure 75. The S protein was detected in cell lysates as a doublet with estimated molecular weight of ~170 ~180 kDa, when the lysate was boiled and analysed under reducing SDS-PAGE conditions (Fig. 75A, lane 3). This doublet appears to result from differential glycosylation of one polypeptide product since pre-treatment of the cell lysate with PNGase F reduced the doublet to a single species of ~140 kDa (Fig. 75A, lane 4). This is the expected size predicted from the aa sequence for a full-length, intact polypeptide product. This experiment indicates that the full length SARS-CoV S is expressed in mammalian cells as a single, uncleaved polypeptide, but in two differentially glycosylated forms, gp170 and gp180 respectively. Unlike the two S glycoforms encoded by the full-length sequence, none of which were secreted, the SΔ protein product was detected both in cell lysates (Fig. 75A, lane 5) as well as in the cell culture medium (Fig. 75B, lane3) as a single species of ~ 160 kDa.

In order to further characterize the intracellular processing of the S protein, and as described above, BHK21 cells were infected with defective alphavirus particles expressing the full-length S. At 6 hr post infection with a MOI of 5, infected cells were pulse labeled for 1 hr with L-[^{35}S] methionine/cysteine and chased for 2 or 4 hours. The [^{35}S]-labeled S protein was immuno-precipitated using the rabbit antiserum raised against inactivated, purified virus and then digested with Endo H. The Endo H treatment involved dilution with a sample buffer (50mM sodium phosphate, 0.1% SDS, 50 mM DTT, pH 6.0) and boiling for 5 min. After denaturation, the samples were further diluted with 0.75% Triton-X 100 and treated with endoglycosidase H (Endo H) following manufacturer's protocol (Calbiochem) for 3 hr at 37°C. Enzyme-treated samples were added with gel loading buffer containing 0.1% SDS and DTT and analyzed by 8% SDS-PAGE.

Both digested and undigested proteins were boiled in SDS and analysed by reducing SDS-PAGE (Figure 55). After a 1-hr pulse, the S protein was apparent as a single gp170 component that was Endo H sensitive (lanes 1 and 2). After a 2-hr chase, a new species (gp180) was present along with gp170 in approximately equal proportions (lane3). After a 4-hr chase, the gp180 species was the dominant S protein component (lane 5) that was now Endo H resistant (lanes 5 and 6). This data is consistent with gp170 being an ER-resident glycoprotein containing high mannose chains and with gp180 corresponding with a Golgi-processed glycoprotein containing Endo H-resistant complex oligosaccharides.

The Endo H sensitivity of the C-terminus deleted SΔ protein purified from cell culture media ws also tested. As shown in Figure 76, the SΔ observed within cell lysates was found to be Endo H sensitive (lanes 1 and 2), while the secreted SΔ in cell culture media was Endo H resistant (lanes 3 and 4). This result is consistent with this glycoprotein being synthesized in an

immature form in the ER prior to transfer to the Golgi where the complex carbohydrate is added and the protein then secreted.

As already described, the S protein expressed in COS7 cells was detected as a gp170/gp180 doublet in western blot analyses of cell lysates that were fully denatured by boiling in the presence of DTT. However, the majority of S protein was detected as a high molecular glycoprotein in the 440-669 kDa range when the same cell lysate was not heat-denatured prior to western blot analysis using SDS-PAGE (Fig. 77, lane 1). The ~500 kDa species was resistant to 10 mM DTT treatment (lane 3) and not dissociated into the monomeric form unless the lysate was first heat-denatured at 100°C (lane 4). In contrast, oligomeric form of a test protein (Thyroglobulin) of which quaternary structure is held by disulfide-linkage was converted into subunit form by the 10 mM DTT treatment. These data suggest that the ~500 kDa oligomeric form of S protein is not disulfide-linked and is heat labile. To confirm the heat-sensitivity of the ~500 kDa species of S protein, the heat-denaturation experiment was repeated but without DTT. As shown in Figure 78, heat denaturation of ~500 kDa protein at 100°C alone was sufficient to convert it into gp170/180 monomeric forms (lane 4). Using a 80°C heat-denaturation step, both the ~500 kDa and monomeric forms were detectable in similar proportion (lane 3).

In order to investigate further whether this ~500 kDa species represents an S protein oligomer in native conformation, comparative analyses with virion-derived S glycoprotein derived from Vero cell cultures was performed. The purified virions were solubilised in 1% SDS prior to Western blot analyses after SDS PAGE. The presence of the ~500 kDa spike protein oligomer was confirmed in virion particles (Fig. 79, lane 1). In addition, heat denaturation of solubilised virions produced the same oligomer-to-monomer conversion as seen with the full-length recombinant S (lanes 2,3). The oligomeric nature of virion S was further analysed in a cross-linking experiment. Aliquots of inactivated virion from sucrose gradient fractions were treated with 10% SDS at 1% final concentration and diluted 2-fold with 0.2M Triethanolamine-HCl (pH 8, Sigma); Dimethyl suberimidate (DMS; Pierce Chemical Co.) was then added from a freshly prepared solution (10mg/ml in 0.2M Triethanolamine-HCl) at 3.3mg/ml final concentration. After 2 hr at room temperature, samples were concentrated with Centricon-30 and analyzed by silver staining after electrophoresis on a 4% polyacrylamide gel. Both untreated and DMS cross-linked virion proteins were heat-denatured, and the heat effect on the maintenance of oligomer structure was analysed by SDS-PAGE and silver staining (Figure 80). In the absence of cross-linking, heat denaturation resulted in the replacement of the ~500 kD spike protein species with the monomer species. In contrast, in the cross-linked proteins, the levels of the ~500 kD and monomer species did not change significantly after heating. These data support the fact that the ~500 kD protein is an oligomer of S monomer proteins that are bound non-covalently. After cross-linking and boiling, the ~500 kDa species migrated as a somewhat slower diffuse form

than the untreated form. This mobility shift is probably due to a structural change resulting from boiling. In addition, a minor protein species of ~300 kD, which may represent a non-dissociated S dimer, could be seen.

To estimate more precisely the size of the recombinant ~500 kDa S species expressed in COS7 cells, a COS7 cell lysate containing the S protein oligomer was fractionated using size-exclusion column chromatography. The major portion of the ~500 kDa oligomer co-eluted with a 572 kDa marker protein. Taken together, these experiments suggest that the ~500 kDa S species seen in COS7 cell lysates is probably a homotrimer of the S protein monomer.

The oligomeric status of the SΔ spike protein was also examined after expression in COS7 cells. As shown in Figure 81, the recombinant SΔ proteins present in cell lysates were also detected in high molecular weight forms of ~500 kDa range when the lysate was not heated prior to SDS-PAGE and Western blot analysis (lane 1). However, the efficiency of oligomerization by intracellular SΔ protein appears to be much less (<10%) compared to that of full-length S protein under the same western analysis conditions. A heat-sensitivity test on this ~500 kDa protein showed that the SΔ oligomer was more heat labile than that of the full-length S oligomer, as demonstrated by the >90% conversion of all of the ~500 kDa species into monomeric Sd forms at 80°C (lane 2). Also (Figure 82), the majority of the secreted SΔ protein was found in monomeric form with the ~500 kDa species barely detectable (and only detectable when the protein was loaded in excess for Western analysis) (lane 1). At a temperature above 80°C, all secreted SΔ proteins were detected as monomers (lanes 2, 3).

The ~500kDa protein is glycosylated, and the effect of deglycosylation on its antibody binding was investigated. The recombinant COS7 lysate was treated with PNGase F under non-denaturing condition (as described above) and analysed by western blot. As shown in Figure 83, deglycosylation did not affect the binding of anti-histidine Mab antibody to the treated S oligomer (lanes 2,3). However, it compromised the reactivity with the rabbit antisera raised against purified virus (lane 6). This antiserum binds to virion-derived S in western blot analyses only when DTT is omitted from the sample for SDS-PAGE indicating that it recognizes primarily a discontinuous, conformational epitope(s). This antisera has also been shown to have a high-titer of viral neutralizing antibodies. Its lack of binding to deglycosylated, recombinant S suggests that the carbohydrate actively contributes to the higher order, native structure of the S polypeptide oligomer.

The difference between the recombinant S and SΔ protein is the presence or absence of the TM-and Cys-rich domains at the C-terminus. This difference predicts that full-length S would be found associated with the membrane fraction while Sd would be in the soluble fraction upon lysis of transfected cells. Therefore, nSh- or nShΔTC-transfected cells were lysed under hypotonic conditions and the soluble cytosol fraction was separated from the insoluble

membrane fraction by centrifugation (Figure 48). As shown in Figure 84, the S protein was found in the membrane fraction (DF) both as a ~500 kDa and 180/170 kDa species (lane 4) but was not detectable in the soluble cytosol fraction (AF) (lane 3). However, the truncated SΔ protein was found as a monomeric species (gp170) in both fractions (lanes 5,6). This indicates that the C-terminal TM and Cys-rich domains are required for the anchorage of the S protein to cell membrane.

The cellular location of the S and SΔ proteins in COS7 cells was analyzed by indirect immunofluorescence microscopy. At 48 hr post-transfection, cells were directly fixed with 2% paraformaldehyde without detergent for cell surface staining or treated with detergent followed by Cytofix/Cytoperm solution for intracellular staining. Fixed cells were then stained with rabbit anti-SARS sera (2BE) and FITC-conjugated antibody. The nSh-transfected cells showed foci of S protein indicative of Golgi-localisation (Figure 85A), while the nShΔTC-transfected cells displayed a uniform distribution of SΔ protein throughout the cytoplasm indicative of ER localisation (Figure 85B). While the complete S protein was also observed on the surface of transfected cells in unfixed cells (Figure 85D), the SΔ was undetectable on the cell surface (Figure 85E). These results indicate the role played by the TM-and Cys-rich domains in anchoring the S protein to the plasma membrane. Although the TM-region alone is likely responsible for membrane anchorage, the potential role played by the Cys-rich region remains to be determined.

The SARS recombinant full-length S protein is thus an N-linked glycoprotein with an estimated molecular weight of 170-180,000 kDa. Deglycosylation with PNGase F resulted in a polypeptide of the expected size for the uncleaved, encoded polypeptide (140kDa). Both transient and stable expression of the full-length SARS-CoV S gene in a variety of mammalian cells, including COS7, 293, BHK21, and Huh7 cell lines, consistently produced a S protein doublet (gp170/180) as detected in western blot analyses. Pulse-chase analyses of transfected cells demonstrated that the SARS CoV S protein was initially synthesized as an Endo H sensitive gp170 species followed by the gradual appearance of an Endo H resistant gp180 form, presumably as a result of the addition of complex carbohydrate within the Golgi apparatus.

The recombinant S protein was not secreted into the cell culture medium unless the C-terminal 60 amino acids containing the TM-region and the Cys-rich tail were deleted.

The quaternary structure of the full-length recombinant S protein was investigated using cross-linking treatment, heat-denaturation, and size fractionation analyses. The results data are consistent with the recombinant S protein existing as a homotrimer of ~500kDa. Similar analyses of virion-derived S yielded the same results. Such a trimeric structure has been reported for other enveloped RNA viruses: the hemagglutinin HA of influenza virus, the E1-E2 heterodimer of alphaviruses and the G protein of vesicular stomatitis virus. Incubations under reducing

conditions indicate that the SARS-CoV S trimeric structure is non-covalently associated, and is very stable. S oligomers present in the cell lysate were shown to be resistant to reduction by 10 mM DTT, detergent treatment with 1% SDS, and heat denaturation at up to 60°C . Incubation at a temperature higher than >80°C resulted in the dissociation of the trimeric complex as evidenced by the decrease in trimer with the concomitant increase in the monomer bands. The temperature-induced appearance of the high-mannosylated gp170 (ER monomer form) as well as the complex-glycosylated gp180 (Golgi monomer form) suggests that trimerization can occur before the transport of the monomer spike protein to the medial Golgi apparatus. This is consistent with other reports for TGEV, influenza virus HA, and vesicular stomatitis virus G proteins. With these proteins, trimerization was reported to take place before addition of complex oligosaccharides in the Golgi apparatus.

The C-terminally truncated form of S was found in the cell lysate in both oligomeric and monomeric forms at a frequency of 10% and 90%, respectively. The truncated protein secreted into medium was found fully glysosylated and it was essentially all in monomeric form. We conclude that the C-terminal 60 amino acids of the S glycoprotein contains a membrane anchor region that affects the efficiency of trimerization. In S protein trimerization, it is possible that the C-terminal region is required to initiate the event and the triple-stranded coiled coil structures in the S2 stalk domain provide further stabilizing force as seen in HA oligomer of influenza virus.

EXAMPLE 24: CHO cells for Spike protein expression

CHO cell lines that stably express either the full-length or truncated SARS-CoV spike proteins were prepared. Several stably transfected CHO cell lines were obtained, and Figure 73 shows western blot data from a panel of representative clones.

EXAMPLE 25: Expression in E.coli

All SARS-CoV ORFs (Figure 17, Table 10) were cloned in the pET vector and expressed as C-terminal His-Tag fusion proteins in *E.coli*. The proteins smaller than 16KD were also expressed as N-terminal GST (Glutathione S-transferase) fusion proteins using pGEX vector.

Nsp1 and Nsp2, the two SARS-CoV proteins with proteolytic activity, were not expressed as full length proteins due to toxicity in *E.coli*. The respective genes were instead cloned in different portions in order to separate the catalytic residues (Cys833/ His994 for Nsp1; His41/Cys145 for Nsp2) in the resulting recombinant proteins: Nsp1A from nucleotides 2719-5214 of AY310120; Nsp1B from nucleotides 5218-7371; Nsp1C from nucleotide 7372-9984; Nsp2A from nucleotide 9985-10416; Nsp2B from nucleotide 10476-10902.

Nsp9 (SEQ ID NO: 9775) was divided into two portions: Nsp9A from nucleotide 13371- 14756; Nsp9B from nucleotide 14757-16166.

Matrix (M), ORF3 and ORF7 contain respectively three, two and one transmembrane domains. These proteins were expressed as deletion proteins excluding the first 100 amino acids (M and ORF3) or the first 18 amino acids (ORF7) that include the hydrophobic regions.

The cloned sequences are shown in Table 26.

A two-step strategy was used to amplify the cloned sequences. In the first step, amplification of DNA fragments containing more than one gene or single gene used sequenced cDNA as template. Eleven cDNA sequences were amplified: (1) a fragment, named amplC1, including genes coding for protein E, protein M, orf 7-8-9-10; (2) a fragment, named amplC2, including genes coding for orf 3-4; (3) a fragment, named amplC5, including genes coding for proteins Nsp12 and Nsp13; (4) Nsp11gene; (5) P28 and P65 genes; (6) Nsp1B and Nsp1C genes portion; (7) a fragment, named amplC9, including genes coding for proteins Nsp2 and Nsp3; (8) a fragment, named amplNsp4-7, including genes coding for proteins Nsp4, Nsp5, Nsp6, Nsp7 and for amplification of Nsp9A gene portion; (9) Nsp 9B gene portion and Nsp10 gene; (10) a fragment, named amplCO, including genes coding for proteins Orf11, Nucleocapsid (N) and Orf12; (11) Nsp1A gene portion. The primers used in this first step are given in Table 27:

In the second step, amplification of single genes was performed using DNA fragments from the first amplification step as templates. The primers are shown in Table 28.

Of the proteins where expression was seen, it was either in inclusion bodies (insoluble) or in a soluble form. Purification proceeded on appropriate material. Table 29 shows the molecular weight of the expressed fragments of SARS-CoV ORFs, whether they were cloned (+ or –), whether the cloned fragment was seen to be expressed (+ or –) and the form of protein which was chosen for purification.

Where a protein was a soluble His-tagged product, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 μg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 μg/ml) liquid medium and grown overnight at 37°C with shaking. The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 μg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD550nm reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0 mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000 x *g* for 15 min at 4°C. The bacterial pellet was resuspended in 10 ml of cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0). Cells were disrupted by sonication (or French Press) on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13 000xg for 30 min at 4°C. Supernatants were mixed with 150μl Ni^{2+}-resin (previously equilibrated with buffer A) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700 x *g* for 5 min at 4°C

and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml buffer A for 10 min, resuspended in 1.0 ml buffer A and loaded onto a disposable column. The resin continued to be washed with buffer A at 4°C until the OD280nm of the flow-through reached 0.02-0.01. The resin was further washed with cold buffer B(300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8.0) until the the OD280nm of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700μl of cold elution buffer C (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) and fractions collected until the OD_{280nm} indicated all the recombinant protein was obtained. 20μl aliquots of each elution fraction were analyzed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

Where a protein was seen as an insoluble product, the inclusion bodies were purified as follows: homogenize cells (5 g wet weight) in 25 ml 0.1M Tris HCl pH 7, 1mM EDTA, at 4°C using an ultraturrax (10000 rpm); add 1.5mg lysozyme per gram cells; mix shortly with an ultraturrax and incubate at 4°C for 30'; use sonication or high-pressure homogenization to disrupt the cells; to digest DNA, add $MgCl_2$ to a final concentration of 3mM and DNase to a final concentration of 10ug/ml and incubate 30' at 25°C. add 0.5 vol of 60mM EDTA, 6% Triton x-100, 1.5M NaCl pH 7.0 to the solution, and incubate for 30' at 4°C; centrifugation at 31000 g for 10' at 4°C; re-suspend pellet in 40ml of 0.1M Tris HCl pH 7.0, 20 mM EDTA using ultraturrax; centrifugation at 31000 g for 10' a 4°C; store the IB pellet at – 20°C.

The results of expression are shown in Figures 86 to 105. Examples of purity and yield are given in Table 30.

EXAMPLE 26: Retention of critical epitope on truncated Spike antigen

A human monoclonal antibody having neutralizing activity was tested in an ELISA assay for reactivity with the purified truncated Spike protein. Briefly, ELISA plates were coated with truncated form of the spike protein at a concentration of 1μg/ml (100μl/ well) by incubating the plates overnight at 4°C. The plates were washed, non-specific binding sites were blocked and then different dilutions of the antibody were added and plates were incubated for 1 hour at room temperature. At the end of incubation, the plates were washed and bound antibody was detected by using anti-human IgG conjugated to horse radish peroxidase (HRP) and an appropriate substrate. The optical density of each well was recorded at 405 nm using an ELISA reader. The data are shown in Figure 69 and clearly demonstrate that the neutralizing epitope recognized by the mAb is preserved and exposed on the recombinant truncated Spike protein.

EXAMPLE 27: Different Spike vaccines

Purified truncated spike protein was used to immunize mice and the level of binding antibodies induced against the truncated spike protein was determined by ELISA assay. Briefly a group of 10 mice were immunized with 3μg of truncated spike protein adjuvanted in MF59 at 0,

4 and 8 weeks intervals. Sera samples were collected from these animals and assayed for antibodies induced by truncated spike protein in an ELISA assay. An additional group of 8 mice was immunized with 75 μg of DNA encoding the truncated form of the spike protein on PLG particles at 0, 4 and 13 weeks intervals, the sera were collected and analyzed as above for anti-spike antibodies as above

The profile of binding antibodies induced in each group was plotted as geometric mean titer (GMT). Compared to a plasmid DNA vaccine expressing truncated spike antigen and delivered using a PLG microparticle formulation, the purified truncated spike protein was significantly more potent for inducing strong antibody responses. Further comparison with the antibody responses induced by inactivated BPL-SARS-CoV (already shown protective) in the same mouse strain revealed that the magnitude of antibody responses induced by purified truncated spike protein and the inactivated virus vaccine are in the same range (Figure 70).

The neutralization potential of antibodies induced by the recombinant truncated spike protein, or plasmid DNA expressing the same spike antigen, were also evaluated. The GMT values obtained in both groups are shown in Figure 71. From these data, it appears that the purified protein is significantly more effective at inducing neutralizing antibody responses against the SARS-CoV spike. Furthermore, the neutralization titers typically induced by the purified truncated spike protein are comparable to neutralization titers induced by an inactivated SARS-CoV vaccine.

Figure 72 shows a comparison of antibody binding levels (ELISA, X-axis) with neutralization titers (Y-axis). In general there is a very good correlation between the binding and neutralizing antibodies. The bottom-left grouping shows ratios 2 weeks post-3rd immunization with the DNA vaccine; the top-right grouping shows ratios 2 weeks post-2nd immunization with the protein vaccine. Both forms of vaccine show a consistent correlation.

In further experiments, the ability of a DNA vaccine to invoke an immune response in mice was studied. Mice were immunized with pCMV-nSdTC plasmid, either free or with PLG microparticles. Serum from the mice was then used as the staining antibody against cultured 293 cells that had been transfected with spike, either full-length or truncated. The cells were centrifuged prior to testing and the pellet was lysed. The antibody was tested against the culture supernatant and against the cell lysate. As shown in Figure 112, the mouse serum was able to detect spike protein in the lysate of cells that expressed full-length spike and in the supernatant of cells that expressed the truncated spike protein. Results were comparable to the staining seen when using rabbit serum that had been obtained after immunization with whole killed virus. Thus anti-spike antibodies can be induced by the use of DNA vaccination.

EXAMPLE 28: Expression cassettes in pCMV

The sequence of plasmid pCMVKm2 is given as SEQ ID NO: 9923. Genes encoding the spike protein either in full-length form (pCMVKm2 SARS Spike nS; SEQ ID NO: 9921) or in its ΔTC form (pCMVKm2 SARS Spike nSΔTC; SEQ ID NO: 9922) were inserted into this basic vector.

Mice were immunized with these vectors, and with similar vectors encoding the N, M or E proteins. Vectors encoding the same proteins but with optimized codon usage were also prepared. Codons were optimized for efficient human expression starting from the FRA sequence (GenBank: AY310120). The optimized sequences are: N (SEQ ID NO: 9924); M (SEQ ID NO: 9925); E (SEQ ID NO: 9926).

After administration, expression of proteins could be detected by immunofluorescence in all cases. For example, Figure 106 shows immunofluorescence (using anti-SARS rabbit serum) results after administration of the vector encoding optimsed N antigen, revealing high level expression. Mice receiving the control vector alone showed no fluorescence.

Figure 107 compares immunofluorescence (using Abgent anti-M antibody) of the native M sequence (107A) or the codon-optimsed M sequence (107B). Similarly, Figure 108 compares immunofluorescence (using Abgent anti-E antibody) of the native E sequence (108A) or the codon-optimsed E sequence (108B).

Four groups of mice (8 mice per group) were immunized with: (1) SARS nS Spike, nSdTC truncated Spike, and N proteins; (2) pCMV-SARS-nSdTC: DNA+DNA-PLG at weeks 0,4 and 13 wks; (3) CMV-nS: DNA+DNA-PLG+VEE/SIN Rep at 0, 4 and 9 wks; (4) VEE/SIN Rep-SARS-nS three times at 0, 4 and 13 wks. Sera from all groups recognized SARS nS and nSdTC proteins, and also showed virus binding and neutralization activity.

EXAMPLE 29: Spike protein cleavage

To investigate the effect of proteolytic cleavage on SARS-CoV Spike protein, it was expressed in various forms in *E.coli*, including: (1) full-length S1-S2; (2) S1 alone; (3) HR1 heptad; and (4) HR2 heptad. The expressed proteins were used to raise immune rabbit sera, which were then used for visualizing western blots of Vero cells, either infected or not infected with SARS-CoV.

Figure 109 shows a western blot using a 1:10000 dilution of antibody raised against either the S1 domain or the uncleaved S1-S2 domains. Figure 110 shows a western blot using a 1:10000 dilution of antibody raised against each of the four proteins. The difference in antigen reactivity is readily apparent.

Figure 111 shows similar data. Each serum was tested against four lanes, with those gour lanes being from left to right: (a) serum at 1:500 dilution, SARS-CoV-infected cells; (b) serum at

1:500 dilution, non-infected cells; (c) serum at 1:2500 dilution, SARS-CoV-infected cells; (d) serum at 1:2500 dilution, non-infected cells. Again, the difference in antigen reactivity is readily apparent.

Figures 109-111 show that the Spike protein exists in various forms in infected Vero cells, with sizes of approx. 75kDa, 90kDa, 180kDa and >250kDa. The Spike protein is cleaved (at least partially) either intracellulary or after release of the particles.

If enzymatic cleavage of the mouse hepatitis coronavirus spike protein is inhibited then cell-cell fusion (syncytia formation) is also inhibited, but virus-cell fusion is not (de Haan *et al.* (2004) *J Virol*). Syncytia are observed *in vivo* in the lungs of SARS-infected patients, but are not seen in Vero cell cultures of the SARS-CoV. Inhibition of Spike protein cleavage may thus be used to prevent syncytia formation and related pathology, even though viral infectivity may not be blocked.

Example 30: Purification of SARS protease

Cells were grown at 37°C to mid-log phase and induced with 0.2% L-arabinose. Cells were harvested by centrifugation, and the cells resuspended in lysis buffer (LB) containing 20 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol V/V, 0.05% Triton X-100, 5 mM βME, 5 mM imidazole, and complete protease inhibitors (–)EDTA. Benzonase was added to a final concentration of 50U/ml of lysate. Cells were then lysed using two passes through a pre-chilled microfluidizer. The lysate was clarified by high speed centrifugation at 44,000 x *g*. Clarified lysate was applied to a prepared Pharmacia chelating FF column charged with nickel sulfate. After application of the lysate the column was washed with 5 column volumes of LB, followed by 5 column volumes of LB supplemented with 45 mM imidazole. The column was then eluted using LB supplemented with 250 mM imidazole. Purity of the isolated SARS protease was 50%. Fractions containing protease were pooled, adjusted to 5 mM EDTA, and then applied to a Superdex 200 gel filtration column equilibrated in 20 mM Tris pH 7.5, 150 mM NaCl, 5% V/V glycerol, 0.05 % Triton X-100, and 5 mM DTT. Purity of the isolated SARS protease was 70%. Again, fractions containing the protease were pooled, and then stored at -80°C until used. Activity assay, mass spectrometry and western blot analysis were used to positively identify the protein (FIG 133). All steps were carried out with pre-chilled buffers, and kept at 4°C for as much of the preparation as possible.

<u>Western of SARS Protease Purification Fractions</u>

Protocol: Briefly, protein concentration was based on Absorbance at 280 nm, and coomassie stained gel estimates of purity. Protein was run on a 4-20% gradient gel, and transferred to nitrocellulose. The blot was then blocked with 3% BSA, probed with Mouse IgG anti-pentaHis,

and then probed with a secondary antibody to Mouse IgG conjugated with HRP. The blot was visualized using an ECL kit (Pharmacia Biotech). The results are shown in Figure 133 where A is the sizing column pool loaded at 50, 100 and 200 ng of target protein and B is the immobilized metal affinity column pool loaded at 50, 100 and 200 ng of target protein.

Example 31: Continuous Fluorescence Resonance Energy Transfer (FRET) Enzyme Assay
The peptide containing EDANS, the fluorescence donor, and DABCYL, the fluorescence quencher (DABCYL-*VNSTLQ* ∇*SGLRK*-EDANS) was synthesized by Syn. Pep. (Dublin, CA). The peptide contains the cleavage site Gln-Ser in the middle. Meyers, G. *et al.* Handbook of Proteolytic Enzymes and Barrett, A *et al.* Academic Press, London, 1998, 726-728. The proteolytic activity of SARS protease was followed kinetically by measuring the level of formation of cleaved product that contains the fluorescence donor, SGLRK-EDANS using the Hitachi fluorometer (F-4500 FL Spec.) set at 340 nm excitation and 490 nm emission wave length. 5 µL of 5 mM peptide stock in DMSO solution was added to the reaction mixture, containing 295 µl of standard buffer (75 mM Tris-Hcl, 25 mM NaOAc, 25 mM Bis-Tris, 25 mM glycine, 5 mM EDTA, and 1 mM EDTA, pH 7.4) and 100 ul of buffer or 100 ul of 3.6 uM protease stock solution. The kinetic curve was followed for 6 minutes (the reaction was linear with R2 value of 0.998 (FIG 134)). The formation of fluorescence (proteolytic reaction) is likely enzyme dependent, as concentration of enzyme was tripled three times as much fluorescence was formed in the 6 minutes time frame.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

Table 1. US Patents and Published International Patent Applications

Publication Number	Title	Publication Date
US-3927216	1,2,4-Triazol E-3-Carboxamides For Inhibiting Virus Infections	12/16/1975
US-4010269	Antiviral Quinazoline Compositions And Methods Of Use	3/1/1977
US-4065570	Antiviral 5-(Substituted Benzal) Hydantoins	12/27/1977
US-4089965	Thiazolylphenylguanidines As Antirhinovirus Agents	5/16/1978
US-4122191	Antirhinovirus Agents	10/24/1978
US-4192895	Antirhinovirus Agents	3/11/1980
US-4254144	Substituted Benzonitriles Having Antiviral Activity	3/3/1981
US-4264617	Antiviral 5-(Substituted Benzal) Hydantoins	4/28/1981
US-4287188	Purine Derivatives	9/1/1981
US-4327088	Phosphonooxy- Or Glycosyloxy-Substituted Acrylophenones, Compositions And Uses Thereof	4/27/1982
US-4332820	Substituted Benzonitriles Having Antiviral Activity	6/1/1982
US-4349568	Sulfur-Substituted Diphenyl Ethers Having Antiviral Activity	9/14/1982
US-4352792	3-Alkoxyflavone Antiviral Agents	10/5/1982
US-4371537	Sulfur-Substituted Phenoxypyridines Having Antiviral Activity	2/1/1983
US-4423053	Derivatives Of 2-Amino-5-(O-Sulphamidophenyl)-1,3,4-Thiadiazol As Antiviral Agents And A Process For The Preparation Thereof	12/27/1983
US-4505929	Sulfur-Substituted Diphenyl Ethers Having Antiviral Activity	3/19/1985
US-4526897	Hypertensive Isoindolin-2-Yl-Aminoimidazolines And Isoindolin-2-Yl-Guanidines	7/2/1985
US-4558134	Certain Phenoxy-Pyridine-Carbonitriles Having Antiviral Activity	12/10/1985
US-4629729	Endowed With Anti-Viral Activity 2-Alkylamino-4,6-Dihalo Pyrimidines	12/16/1986
US-4636492	Inhibition Of Viral Protease Activity By Peptide Halomethyl Ketones	1/13/1987
US-4652552	Tetrapeptide Methyl Ketone Inhibitors Of Viral Proteases	3/24/1987
US-4724233	Therapeutical Application Of Phosphonylmethoxyalkyl Adenines	2/9/1988
US-4738984	Antirhinovirus Agents	4/19/1988
US-4847246	Antiviral Compositions Derived From Fireflies And Their Methods Of Use	7/11/1989
US-4855283	Novel Pharmaceutically Active N-(2-Aminoacylamido-2-Deoxy-Hexosyl)-Amides, -Carbamates And -Ureas	8/8/1989
US-4885285	Phosphorus Compounds, Processes For Their Manufacture, And Their Use	12/5/1989
US-4956351	Antiviral Pharmaceutical Compositions Containing Cyclodextrins	9/11/1990
US-5001125	Anti-Virally Active Pyridazinamines	3/19/1991
US-5036072	Antiviral Agent	7/30/1991
US-5070090	Antipicorpaviral Herterocyclic-Substituted Morpholinyl Alkylphenol Ethers	12/3/1991
US-5100893	Antipicornaviral Pyridazinamines	3/31/1992
US-5112825	Antirhinoviral Heteroamine-Substituted Pyridazines	5/12/1992
US-5157035	Anti-Virally Active Pyridazinamines	10/20/1992
US-5240694	Combined Antiviral And Antimediator Treatment Of Common Colds	8/31/1993
US-5242924	Tetrazolyl-(Phenoxy And Phenoxyalkyl)-Piperidinylpyridazines As Antiviral Agents	9/7/1993
US-5278184	Synthetic Derivatives Of Pyrrole And Pyrrolidine Suitable For The Therapy Of Infections Caused By Rhinoviruses	1/11/1994
US-5364865	Phenoxy- And Phenoxyalkyl-Piperidines As Antiviral Agents	11/15/1994
US-5453433	Thiadiazoles And Antipicornaviral Compositions	9/26/1995
US-5492689	Combined Virustatic Antimediator (COVAM) Treatment Of Common Colds	2/20/1996
US-5514679	Therapeutic Phenoxyalklpyridazines And Intermediates Therefor	5/7/1996
US-5514692	Substituted Quinoline Derivatives Useful As Antipiconaviral Agents	5/7/1996
US-5523312	Antipicornaviral Agents	6/4/1996
US-5545653	Anti-Viral Compounds	8/13/1996
US-5552420	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	9/3/1996
US-5567719	Thiadiazoles And Their Use As Antipicornaviral Agents	10/22/1996

US-5580897	1,2-Dithiins Having Antifungal Activity	12/3/1996
US-5618821	Therapeutic Phenoxyalkylheterocycles	4/8/1997
US-5618849	Orally Active Antiviral Compounds	4/8/1997
US-5648354	1,2-Dithiins Having Antifungal Activity	7/15/1997
US-5650419	Thiadiazoles And Their Use As Antipicornaviral Agents	7/22/1997
US-5693661	Anti-Viral Compounds	12/2/1997
US-5721261	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	2/24/1998
US-5725859	Plant-Based Therapeutic Agent With Virustatic And Antiviral Effect	3/10/1998
US-5750527	Thiadiazoles And Their Use As Antipicornaviral Agents	5/12/1998
US-5750551	Treatment For Viral Diseases	5/12/1998
US-5762940	Methods And Compositions For Inhibiting Or Destroying Viruses Or Retroviruses	6/9/1998
US-5763461	Therapeutic Phenoxyalkylheterocycles	6/9/1998
US-5821242	Anti-Viral Compounds	10/13/1998
US-5821257	Thiadiazoles And Their Uses As Antipicornaviral Agents	10/13/1998
US-5821331	Anti-Picornaviral Agents	10/13/1998
US-5846986	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	12/8/1998
US-5856530	Antipicornaviral Compounds And Methods For Their Use And Preparation	1/5/1999
US-5891874	Anti-Viral Compound	4/6/1999
US-5962487	Antipicornaviral Compounds And Methods For Their Use And Preparation	10/5/1999
US-6004933	Cysteine Protease Inhibitors	12/21/1999
US-6020371	Antipicornaviral Compounds Compositions Containing Them And Methods For Their Use	2/1/2000
US-6087374	Anti-Viral Compounds	7/11/2000
US-6114327	Anti-Viral Compounds	9/5/2000
US-6117844	Method And Composition For Antiviral Therapy	9/12/2000
US-6194447	Bis (Benzimidazole) Derivatives Serving As Potassium Blocking Agents	2/27/2001
US-6214799	Antipicornaviral Compounds And Methods For Their Use And Preparation	4/10/2001
US-6277891	Nitric Oxide Inhibits Rhinovirus Infection	8/21/2001
US-6294186	Antimicrobial Compositions Comprising A Benzoic Acid Analog And A Metal Salt	9/25/2001
US-6331554	Antipicornaviral Compounds, Compositions Containing Them, And Methods For Their Use	12/18/2001
US-6358971	Anti-Viral Compounds	3/19/2002
US-6362166	Antipicornaviral Compounds And Methods For Their Use And Preparation	3/26/2002
US-6414004	3-Substituted 5-Aryl-4-Isoxazolecarbonitriles Having Antiviral Activity	7/2/2002
US-6420591	Carbamates And Compositions Thereof, And Methods For Their Use For Treating Cancer, Inflammation, Or A Viral Infection	7/16/2002
US-6469018	Compounds	10/22/2002
US-6498178	Inhibitors Of IMPDH Enzyme	12/24/2002
US-6514997	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	2/4/2003
US-6525043	Use Of Ion Channel Modulating Agents	2/25/2003
US-6531452	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	3/11/2003
US-6534489	Organophosphorus Compounds And The Use Thereof	3/18/2003
WO00/06529	Diketoacid-Derivatives As Inhibitors Of Polymerases	2/10/2000
WO00/25791	Pyridin-4-Yl Or Pyrimidin-4-Yl Substituted Pyrazines	5/11/2000
WO00/27423	Methods And Compositions For Treating Common Cold Symptoms	5/18/2000
WO00/34308	Protein Transduction System And Methods Of Use Thereof	6/15/2000
WO00/39348	Methods And Compositions For Identifying Protease Modulators	7/6/2000
WO00/40243	Novel Compounds	7/13/2000
WO00/50037	Nitrosated And Nitrosylated Proton Pump Inhibitors, Compositions And Methods Of Use	8/31/2000
WO00/56331	Inhibitors Of Impdh Enzyme	9/28/2000
WO00/56757	Immunomodulatory Steroids, In Particular The Hemihydrate Of 16.Alpha.-Bromoepiandrosterone	9/28/2000
WO00/66096	New Indication For Use Of Antiepileptic Agents And Medicines	11/9/2000
WO00/78746	Antiviral Agents	12/28/2000

WO01/00199	Compounds Obtained From Salvia Species Having Antiviral Activity	1/4/2001
WO01/00585	Pyrazolidinol Compounds	1/4/2001
WO01/02551	Virus Like Particles, Their Preparation And Their Use Preferably In Pharmaceutical Screening And Functional Genomics	1/11/2001
WO01/03681	Use Of Flavones, Coumarins And Related Compounds To Treat Infections	1/18/2001
WO01/05396	Use Of Cobalt Chelates For Treating Or Preventing Virus Infection	1/25/2001
WO01/10894	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	2/15/2001
WO01/19322	Use Of Csaids In Rhinovirus Infection	3/22/2001
WO01/19822	Antiviral Agents	3/22/2001
WO01/22920	Colon And Colon Cancer Associated Polynucleotides And Polypeptides	4/5/2001
WO01/25188	Novel Carbamates And Ureas	4/12/2001
WO01/31016	Processed Human Chemokines Phc-1 And Phc-2	5/3/2001
WO01/37837	3,4-Dihydro-(1h)-Quinazolin-2-Ones And Their Use As Csbp/P38 Kinase Inhibitors	5/31/2001
WO01/38312	3,4-Dihydro-(1h)Quinazolin-2-One Compounds As Csbp/P38 Kinase Inhibitors	5/31/2001
WO01/38313	3,4-Dihydro-(1h)Quinazolin-2-One Compounds As Csbp/P39 Kinase Inhibitors	5/31/2001
WO01/38314	3,4-Dihydro-(1h)Quinazolin-2-One Compounds As Csbp/P38 Kinase Inhibitors	5/31/2001
WO01/40189	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	6/7/2001
WO01/49303	Multivalent Electron Active Compositions And Methods Of Making And Using Same	7/12/2001
WO01/60393	Selective Destruction Of Cells Infected With Human Immunodeficiency Virus	8/23/2001
WO01/62726	2-Oxo-1-Pyrrolidine Derivatives, Processes For Preparing Them And Their Uses	8/30/2001
WO01/79167	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	10/25/2001
WO01/90047	Novel Mmp-2/Mmp-9 Inhibitors	11/29/2001
WO01/90129	Prophylactic And Therapeutic Treatment Of Infectious And Other Diseases With Mono- And Disaccharide-Based Compounds	11/29/2001
WO01/92499	Nucleic Acid Molecules Encoding A Protein Interacting With Ser/Thr Kinase Akt	12/6/2001
WO01/93883	Therapeutic Agents - Iii	12/13/2001
WO01/93884	Therapeutic Agents - I	12/13/2001
WO01/93885	Therapeutic Agents - Ii	12/13/2001
WO01/96297	Antipicornaviral Compounds And Compositions, Their Pharmaceutical Uses, And Materials For Their Synthesis	12/20/2001
WO02/04413	Chiral Integrin Modulators And Methods Of Use Thereof	1/17/2002
WO02/11743	Treatment Of Prostate Cancer	2/14/2002
WO02/12477	Enhanced Replication Of Hcv Rna	2/14/2002
WO02/14343	Immunosuppressive, Antiinflammatory And Analgetic Compounds	2/21/2002
WO02/24145	Antiviral Substances From Plant Cuticular And Epicuticular Material	3/28/2002
WO02/28351	Recombinant Mucin Binding Proteins From Steptococcus Pneumoniae	4/11/2002
WO02/30442	Method For Treating Cytokine Mediated Hepatic Injury	4/18/2002
WO02/34771	Nucleic Acids And Proteins From Streptococcus Groups A & B	5/2/2002
WO02/44737	Diagnostic And Therapeutic Compositions And Methods Related To G Protein-Coupled Receptor (Gpcr) Anaphylatoxin C3a Receptor	6/6/2002
WO02/50045	Antiviral Agents	6/27/2002
WO02/51413	Macrocyclic Anti-Viral Compounds	7/4/2002
WO02/53138	Treatment For Inhibiting Neoplastic Lesions	7/11/2002
WO02/57425	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/59083	Novel Compounds	8/1/2002
WO02/60875	Nicotinamide Biaryl Derivatives Useful As Inhibitors Of Pde4 Isozymes	8/8/2002
WO02/60898	Thiazolyl-, Oxazolyl-, Pyrrolyl-, And Imidazolyl-Acid Amide Derivatives Useful As Inhibitors Of Pde4 Isozymes	8/8/2002
WO02/69903	Nucleosides, Preparation Thereof And Use As Inhibitors Of Rna Viral Polymerases	9/12/2002
WO02/72022	Substituted Tetracycline Compounds As Antifungal Agents	9/19/2002

WO02/72031	Substituted Tetracycline Compounds As Synergistic Antifungal Agents	9/19/2002
WO02/76939	Cysteine Protease Inhibitors	10/3/2002
WO02/77021	Streptococcus Pneumoniae Proteins And Nucleic Acids	10/3/2002
WO02/79401	Novel Rgs9 Protein Binding Interactions And Methods Of Use Thereof	10/10/2002
WO02/82041	Production And Use Of Novel Peptide-Based Agents For Use With Bi-Specific Antibodies	10/17/2002
WO02/87465	Compositions And Methods Of Double-Targeting Virus Infections And Cancer Cells	11/7/2002
WO02/87500	Viral Enzyme Activated Prototoxophores And Use Of Same To Treat Viral Infections	11/7/2002
WO02/88091	Inhibitors Of Human Rhinovirus 2a Cysteine Protease	11/7/2002
WO02/89832	Pharmaceutical Compositions For Preventing Or Treating Th1 And Th2 Cell Related Diseases By Modulating The Th1/Th2 Ratio.	11/14/2002
WO02/92779	Method For Enriching Tissues In Long Chain Polyunsaturated Fatty Acids	11/21/2002
WO02/94185	Conjugates And Compositions For Cellular Delivery	11/28/2002
WO02/94868	Staphylococcus Aureus Proteins And Nucleic Acids	11/28/2002
WO02/96867	Inhibitors Of Protein Kinase For The Treatment Of Disease	12/5/2002
WO02/98424	Novel Anti-Infectives	12/12/2002
WO03/04489	Compositions And Methods For Inhibiting Prenyltransferases	1/16/2003
WO03/08628	Enzymatic Nucleic Acid Peptide Conjugates	1/30/2003
WO03/15744	Chitin Microparticles And Their Medical Uses	2/27/2003
WO03/20222	Dioxolane And Oxathiolane Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	3/13/2003
WO03/20270	Oxadiazolyl-Phenoxyalkylisoxazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO03/20271	Oxadiazolyl-Phenoxyalkylisoxazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO03/20712	Oxadiazolyl-Phenoxyalkylthiadiazoles, Compositions Thereof And Methods For Their Use As Anti-Picornaviral Agents	3/13/2003
WO86/03412	Improvements Relating To The Treatment Control And Prevention Of Rhinovirus Infections	6/19/1986
WO86/03971	Antiviral Agents	7/17/1986
WO88/09669	Avirulent Microbes And Uses Therefor	12/15/1988
WO92/03475	Enterovirus Peptides	3/5/1992
WO92/22520	Orally Active Antiviral Compounds	12/23/1992
WO92/22570	Inhibitors Of Picornavirus Proteases	12/23/1992
WO94/00012	Nucleic Acids And Methods Of Use Thereof For Controlling Viral Pathogens	1/6/1994
WO95/03821	Prosaposin And Cytokine-Derived Peptides As Therapeutic Agents	2/9/1995
WO95/09175	Ring-Expanded Nucleosides And Nucleotides	4/6/1995
WO95/11992	Antiviral Compounds	5/4/1995
WO95/31198	Thiadiazoles And Their Use As Antipicornaviral Agents	11/23/1995
WO95/31438	Therapeutic Phenoxyalkylheterocycles	11/23/1995
WO95/31439	Therapeutic Phenoxyalkylpyridazines And Intermediates Therefor	11/23/1995
WO95/31452	Therapeutic Phenoxyalkylazoles And Phenoxyalkylazines	11/23/1995
WO95/34595	Antiviral Dendrimers	12/21/1995
WO95/35103	A Pharmaceutical Composition For The Prevention And/Or Treatment Of Viral Infections And Optionally Inflammations As Well As A Method For The Treatment Thereof	12/28/1995
WO96/05836	Methods Of Treating Cold Symptoms Using Pentoxifylline	2/29/1996
WO96/05854	Combination Preparation, Containing Cyclosporin A Or Fk506 Or Rapamycin And A Xanthine Derivative	2/29/1996
WO96/09822	Antipicornaviral Agents	4/4/1996
WO96/11211	Selective Inhibition Of Internally Initiated Rna Translation	4/18/1996
WO96/22689	Multiple Component Rna Catalysts And Uses Thereof	8/1/1996
WO96/40641	Sulfonamide Derivatives As Cell Adhesion Modulators	12/19/1996
WO97/08553	Targeting Of Proteins To The Cell Wall Of Gram-Positive Bacteria	3/6/1997
WO97/34566	Electrophilic Ketones For The Treatment Of Herpesvirus Infections	9/25/1997
WO97/41137	Use Of Anthocyanidin And Anthocyanidin Derivatives	11/6/1997
WO97/43305	Inhibitors Of Picornavirus 3c Proteases And Methods For Their Use And Preparation	11/20/1997

WO97/47270	Novel Anti-Viral Compounds	12/18/1997
WO98/03572	Antiviral Linear Polymers	1/29/1998
WO98/07745	Compositions And Methods For Treating Infections Using Analogues Of Indolicidin	2/26/1998
WO98/11778	Antimicrobial Treatment For Herpes Simplex Virus And Other Infectious Diseases	3/26/1998
WO98/22495	Antikinin Compounds And Uses Thereof	5/28/1998
WO98/31363	Anti-Viral Compounds	7/23/1998
WO98/31374	Method Of Treating Rhinoviral Infections	7/23/1998
WO98/32427	Therapeutic Treatment And Prevention Of Infections With A Bioactive Material Encapsulated Within A Biodegradable-Biocompatible Polymeric Matrix	7/30/1998
WO98/34601	Method For Inhibiting Intracellular Viral Replication	8/13/1998
WO98/42188	Antimicrobial Prevention And Treatment Of Human Immunedeficiency Virus And Other Infectious Diseases	10/1/1998
WO98/43950	Antipicornaviral Compouds, Compositions Containing Them, And Methods For Their Use	10/8/1998
WO98/49190	Substituted Oxadiazole Cysteine Protease Inhibitors	11/5/1998
WO98/55120	Anti-Viral Compounds	12/10/1998
WO99/30699	Modulators Of Cysteine Protease	6/24/1999
WO99/31122	Antipicornaviral Compounds And Methods For Their Use And Preparation	6/24/1999
WO99/54317	Cysteine Protease Inhibitors	10/28/1999
WO99/55663	Inhibitors Of Impdh Enzyme	11/4/1999
WO99/57135	Antipicornaviral Compounds, Their Preparation And Use	11/11/1999
WO99/59587	Anti-Viral Compounds	11/25/1999
WO99/61437	Novel 2-Alkyl Substituted Imidazole Compounds	12/2/1999

Table 2. US Patents and Published International Patent Applications

Publication Number	Title	Publication Date
WO02/69903	Nucleosides, Preparation Thereof And Use As Inhibitors Of Rna Viral Polymerases	9/12/2002
WO02/48116	Inhibitors Of Hepatitis C Virus Ns3 Protease	6/20/2002
WO02/48157	Imidazolidinones And Their Related Derivatives As Hepatitis C Virus Ns3 Protease Inhibitors	6/20/2002
WO02/61048	In Vitro System For Replication Of Rna-Dependent Rna Polymerase (Rdrp) Viruses	8/8/2002
WO03/02518	Novel 2,4-Difluorobenzamide Derivatives As Antiviral Agents	1/9/2003
WO02/79187	Methoxy-1,3,5-Triazine Derivatives As Antiviral Agents	10/10/2002
WO01/78648	6-Methylnicotinamide Derivatives As Antiviral Agents	10/25/2001
WO01/12214	MYCOPHENOLATE MOFETIL IN ASSOCIATION WITH PEG-IFN-.Alpha.	2/22/2001
WO02/100415	4'-Substituted Nucleosides	12/19/2002
WO02/18404	Nucleoside Derivatives	3/7/2002
WO02/94289	Antiviral Nucleoside Derivatives	11/28/2002
WO96/39500	Oligonucleotides Specific For Hepatitis C Virus	12/12/1996
WO03/00713	Nucleoside Compounds In Hcv	1/3/2003
WO01/60381	Nucleoside Analogs With Carboxamidine-Modified Bicyclic Base	8/23/2001
WO02/03997	Pyrido[2,3-D]Pyrimidine And Pyrimido[4,5-D]Pyrimidine Nucleosides	1/17/2002
WO97/26883	Modulation Of Th1/Th2 Cytokine Expression By Ribavirin3 And Ribavirin3 Analogs In Activated T-Lymphocytes	7/31/1997
WO03/26589	Methods And Compositions For Treating Hepatitis C Virus Using 4'-Modified Nucleosides	4/3/2003
WO03/26675	Methods And Compositions For Treating Flaviviruses And Pestiviruses Using 4'-Modified Nucleoside	4/3/2003
WO97/30067	Sugar-Modified Gapped Oligonucleotides	8/21/1997
WO01/47883	Fused-Ring Compounds And Use Thereof As Drugs	7/5/2001
WO03/00254	Fused Cyclic Compounds And Medicinal Use Thereof	1/3/2003
WO02/100354	Pyrrolo[2,3-D]Pyrimidine Nucleoside Analogs	12/19/2002
WO01/55111	Biaryl Compounds, Their Preparation And Their Use In Therapy	8/2/2001

WO01/16149	2-Azapurine Compounds And Their Use	3/8/2001
WO01/85770	Sentinel Virus Ii	11/15/2001
WO02/12263	Nucleic Acid Binding Compounds Containing Pyrazolo[3,4-D]Pyrimidine Analogues Of Purin-2,6-Diamine And Their Uses	2/14/2002
JP 2001-247550 A2	Condensed Ring Compound And Its Medicinal Use	9/11/2001
6210675	PT-NANB Hepatitis Polypeptides	4/3/2001
6451991	Sugar-Modified Gapped Oligonucleotides	9/17/2002
5830455	Method Of Treatment Using A Therapeutic Combination Of α-Interferon And Free Radical Scavengers	11/3/1998
5908621	Polyethylene Glycol Modified Interferon Therapy	6/1/1999
5990276	Synthetic Inhibitors Of Hepatitis C Virus NS3 Protease	11/23/1999
6172046	Combination Therapy For Eradicating Detectable HCV-RNA In Patients Having Chronic Hepatitis C Infection	1/9/2001
6177074	Polyethylene Glycol Modified Interferon Therapy	1/23/2001
6326137	Hepatitis C Virus Protease-Dependent Chimeric Pestivirus	12/4/2001
6434489	Compositions Of Hepatitis C Virus NS5B Polymerase And Methods For Crystallizing Same	8/13/2002
6461605	Continuous Low-Dose Cytokine Infusion Therapy	10/8/2002
6472373	Combination Therapy For Eradicating Detectable HCV-RNA In Antiviral Treatment Naive Patients Having Chronic Hepatitis C Infection	10/29/2002
6524570	Polyethylene Glycol Modified Interferon Therapy	2/25/2003
WO00/37097	Ribavirin-Interferon Alfa Induction Hcv Combination Therapy	6/29/2000
WO00/37110	Ribavirin-Pegylated Interferon Alfa Induction Hcv Combination Therapy	6/29/2000
WO00/62799	Hcv Combination Therapy, Containing Ribavirin In Association With Antioxidants	10/26/2000
WO01/58929	Azapeptides Useful In The Treatment Of Hepatitis C	8/16/2001
WO02/32414	Ribavirin-Pegylated Interferon Alfa Hcv Combination Therapy	4/25/2002
WO03/24461	Hcv Combination Therapy	3/27/2003
WO93/20835	Treatment Of Hepatitis With Gm-Csf	10/28/1993
WO96/36702	Soluble, Active Hepatitis C Virus Protease	11/21/1996
WO97/16204	Continuous Low-Dose Cytokine Infusion Therapy	5/9/1997
WO97/43310	Synthetic Inhibitors Of Hepatitis C Virus Ns3 Protease	11/20/1997
WO98/48840	Polyethylene Glycol-Interferon Alpha Conjugates For Therapy Of Infection	11/5/1998
WO99/15194	Combination Therapy For Eradicating Detectable Hcv-Rna In Patients Having Chronic Hepatitis C Infection	4/1/1999
WO99/59621	Combination Therapy Comprising Ribavirin And Interferon Alpha In Antiviral Treatment Naive Patients Having G Chronic Hepatitis C Infection	11/25/1999
WO02/100846	Compounds And Methods For The Treatment Or Prevention Of Flavivirus Infections	12/19/2002
WO02/100851	Compounds And Methods For The Treatment Or Prevention Of Flavivirus Infections	12/19/2002
5241053	Fused Proteins Comprising Glycoprotein Gd Of HSV-1 And LTB	8/31/1993
5556946	Interleukin-2/Viral Antigen Protein Chimers	9/17/1996
6087484	Enhancement Of Ribozyme Catalytic Activity By A 2'-O-Substituted Facilitator Oligonucleotide	7/11/2000
5830905	Compounds, Compositions And Methods For Treatment Of Hepatitis C	11/3/1998
6316492	Methods For Treating Or Preventing Viral Infections And Associated Diseases	11/13/2001
6440985	Methods For Treating Viral Infections	8/27/2002
WO00/10573	Compounds, Compositions And Methods For Treating Or Preventing Viral Infections And Associated Diseases	3/2/2000
WO00/13708	Methods For Treating Or Preventing Viral Infections And Associated Diseases	3/16/2000
WO00/18231	Methods For Treating Or Preventing Viral Infections And Associated Diseases	4/6/2000
WO99/51781	Hepatitis C Virus Ns5b Compositions And Methods Of Use Thereof	10/14/1999
6323180	Hepatitis C Inhibitor Tri-Peptides	11/27/2001
6143715	Hepatitis C Inhibitor Peptide Analogues	11/7/2000
6329379	Hepatitis C Inhibitor Tri-Peptides	12/11/2001
6329417	Hepatitis C Inhibitor Tri-Pepitides	12/11/2001

6410531	Hepatitis C Inhibitor Tri-Peptides	6/25/2002
6420380	Hepatitis C Inhibitor Tri-Peptides	7/16/2002
6448281	Viral Polymerase Inhibitors	9/10/2002
6479508	Viral Polymerase Inhibitors	11/12/2002
6534523	Hepatitis C Inhibitor Tri-Peptides	3/18/2003
WO00/09543	Hepatitis C Inhibitor Tri-Peptides	2/24/2000
WO00/09558	Hepatitis C Inhibitor Peptides	2/24/2000
WO00/59929	Macrocyclic Peptides Active Against The Hepatitis C Virus	10/12/2000
WO02/04425	Viral Polymerase Inhibitors	1/17/2002
WO02/70739	Hcv Polymerase Inhibitor Assay	9/12/2002
WO03/07945	Viral Polymerase Inhibitors	1/30/2003
WO03/10140	Viral Polymerase Inhibitors	2/6/2003
WO03/10141	Viral Polymerase Inhibitors	2/6/2003
WO99/07734	Hepatitis C Inhibitor Peptide Analogues	2/18/1999
WO01/16379	Hepatitis C Virus Replication Inhibitors	3/8/2001
WO02/07761	Inhibiting Hepatitis C Virus Processing And Replication	1/31/2002
WO02/57287	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/57425	Nucleoside Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	7/25/2002
WO02/70651	Viral Reporter Particles	9/12/2002
WO03/20222	Dioxolane And Oxathiolane Derivatives As Inhibitors Of Rna-Dependent Rna Viral Polymerase	3/13/2003
PCT/US2003/041493	Thiosemicarbazones as Anti-Virals and Immunopotentiators	01/10/2003

Table 3: US Patents and published international patent applications relating to inhalation technology for the delivery of antiviral compounds of the invention.

Publication Number	Title	Publication Date
5740794	Apparatus and methods for dispersing dry powder medicaments	4/21/1998
5775320	Method and device for delivering aerosolized medicaments	7/7/1998
5785049	Method and apparatus for dispersion of dry powder medicaments	7/28/1998
5814607	Pulmonary delivery of active fragments of parathyroid hormone	9/29/1998
5826633	Powder filling systems, apparatus and methods	10/27/1998
5458135	Method and device for delivering aerosolized medicaments	10/17/1995
5607915	Pulmonary delivery of active fragments of parathyroid hormone	3/4/1997
5654007	Methods and system for processing dispersible fine powders	8/5/1997
5922354	Methods and system for processing dispersible fine powders	7/13/1999
5928469	Process for storage of materials	7/27/1999
5976574	Processes for spray drying hydrophobic drugs in organic solvent suspensions	11/2/1999
5985248	Processes for spray drying solutions of hydrophobic drugs and compositions thereof	11/16/1999
5994314	Compositions and methods for nucleic acid delivery to the lung	11/30/1999
5997848	Methods and compositions for pulmonary delivery of insulin	12/7/1999
6001336	Processes for spray drying aqueous suspensions of hydrophobic drugs and compositions thereof	12/14/1999
6019968	Dispersible antibody compositions and methods for their preparation and use	2/1/2000
6051256	Dispersible macromolecule compositions and methods for their preparation and use	4/18/2000
6071428	Stable compositions	6/6/2000
6077543	Systems and processes for spray drying hydrophobic drugs with hydrophilic excipients	6/20/2000
6080721	Pulmonary delivery of active fragments of parathyroid hormone	6/27/2000
6089228	Apparatus and methods for dispersing dry powder medicaments	7/18/2000
6103270	Methods and system for processing dispersible fine powders	8/15/2000
6123936	Methods and compositions for the dry powder formulation of interferons	9/26/2000

6136346	Powdered pharmaceutical formulations having improved dispersibility	10/24/2000
6138668	Method and device for delivering aerosolized medicaments	10/31/2000
6165463	Dispersible antibody compositions and methods for their preparation and use	12/26/2000
6182712	Power filling apparatus and methods for their use	2/6/2001
6187344	Powdered pharmaceutical formulations having improved dispersibility	2/13/2001
6207135	Gaseous microparticles for ultrasonic diagnosis and process for their production	3/27/2001
6231851	Methods and compositions for the dry powder formulation of interferons	5/15/2001
6257233	Dry powder dispersing apparatus and methods for their use	7/10/2001
6258341	Stable glassy state powder formulations	7/10/2001
6267155	Powder filling systems, apparatus and methods	7/31/2001
6294204	Method of producing morphologically uniform microcapsules and microcapsules produced by this method	9/25/2001
6303582	Compositions and methods for nucleic acid delivery to the lung	10/16/2001
6309623	Stabilized preparations for use in metered dose inhalers	10/30/2001
6309671	Stable glassy state powder formulations	10/30/2001
6358530	Powdered pharmaceutical formulations having improved dispersibility	3/19/2002
6365190	Systems and processes for spray drying hydrophobic drugs with hydrophilic excipients	4/2/2002
6372258	Methods of spray-drying a drug and a hydrophobic amino acid	4/16/2002
6423344	Dispersible macromolecule compositions and methods for their preparation and use	7/23/2002
6426210	Storage of materials	7/30/2002
6433040	Stabilized bioactive preparations and methods of use	8/13/2002
6440337	Method and apparatus for the formation of particles	8/27/2002
RE37872	Storage of materials	10/8/2002
6479049	Methods and compositions for the dry powder formulation of interferons	11/12/2002
6503411	Stable compositions	1/7/2003
6509006	Devices compositions and methods for the pulmonary delivery of aerosolized medicaments	1/21/2003
6514496	Dispersible antibody compositions and methods for their preparation and use	2/4/2003
6518239	dry powder compositions having improved dispersivity	2/11/2003
6543448	apparatus and methods for dispersing dry powder medicaments	4/8/2003
6546929	dry powder dispersing apparatus and methods for their use	4/15/2003
WO 00/15262	dry powder active agent pulmonary delivery	3/23/2000
WO 93/00951	method and device for delivering aerosolized medicaments	1/21/1993
WO 94/07514	pulmonary delivery of active fragments of parathyroid hormone	4/14/1994
WO 95/24183	methods and compositions for pulmonary delivery of insulin	9/14/1995
WO 95/31479	methods and compositions for the dry powder formulation of interferons	11/23/1995
WO 96/09085	apparatus and methods for dispersing dry powder medicaments	3/28/1996
WO 96/32096	powdered pharmaceutical formulations having improved dispersibility	10/17/1996
WO 96/32116	compositions and methods for nucleic acid delivery to the lung	10/17/1996
WO 96/32149	pulmonary delivery of aerosolized medicaments	10/17/1996
WO 96/32152	pulmonary administration of dry powder alpha 1-antitrypsin	10/17/1996
WO 96/40068	methods and system for processing dispersible fine powders	12/19/1996
WO 97/41031	powder filling systems, apparatus and methods	11/6/1997
WO 97/41833	dispersible macromolecule compositions and methods for their preparation and use	11/13/1997
WO 98/16205	stable glassy state powder formulations	4/23/1998
WO 98/29096	aerosolized hydrophobic drug	7/9/1998
WO 98/29098	processes for spray drying aqueous suspensions of hydrophobic drugs with hydrophilic excipients and compositions prepared by such processes	7/9/1998
WO 98/29140	processes and compositions for spray drying hydrophobic drugs in organic solvent suspensions of hydrophilic excipients	7/9/1998
WO 98/29141	processes for spray drying solutions of hydrophobic drugs with hydrophilic excipients and compositions prepared by such processes	7/9/1998
WO 99/19215	powder filling apparatus and method	4/22/1999
WO 99/42124	liquid crystal forms of cyclosporin	8/26/1999

WO 99/47196	aerosolized active agent delivery	9/23/1999
WO 99/62495	dry powder dispersing apparatus and methods for their use	12/9/1999
WO 00/21594	flow resistance modulated aerosolized active agent delivery	4/20/2000
WO 00/61178	pulmonary administration of dry powder formulations for treating infertility	10/19/2000
WO 00/72904	apparatus and method for dispensing metered amount of aerosolized medication	12/7/2000
WO 01/00263	systems and methods for aerosolizing pharmaceutical formulations	1/4/2001
WO 01/00312	spray drying process for preparing dry powders	1/4/2001
WO 01/32144	dry powder compositions having improved dispersivity	5/10/2001
WO 01/43529	receptacles to facilitate the extraction of powders	6/21/2001
WO 01/43530	systems and methods for extracting powders from receptacles	6/21/2001
WO 01/43802	systems and methods for treating packaged powders	6/21/2001
WO 01/44764	systems and methods for non-destructive mass sensing	6/21/2001
WO 01/87393	systems, devices and methods for opening receptacles having a powder to be fluidized	11/22/2001
WO 01/93932	lockout mechanism for aerosol drug delivery devices	12/13/2001
WO 02/09669	apparatus and process to produce particles having a narrow size distribution and particles made thereby	2/7/2002
WO 02/11695	inhaleable spray dried 4-helix bundle protein powders having minimized aggregation	2/14/2002
WO 02/49619	induced phase transition method for the production of microparticles containing hydrophilic active agents	6/27/2002
WO 02/49620	induced phase transition method for the production of microparticles containing hydrophobic active agents	6/27/2002
WO 02/54868	pulmonary delivery of polyene antifungal agents	7/18/2002
WO 02/87542	novel methods and compositions for delivering macromolecules to or via the respiratory tract	11/7/2002
WO 02/100548	centrifuged rotating drum for treating cohesive powders	12/19/2002
WO 03/00326	powder aerosolization apparatus and method	1/3/2003
WO 03/00329	flow regulator for aerosol drug delivery device and methods	1/3/2003

TABLE 4: Forward and reverse primers for nucleic acid amplification of SARSV

Pair Number	Forward primer SEQ ID NO	Forward Primer Start	Forward Primer Stop	Forward Primer Tm	Forward Primer %GC	Reverse primer SEQ ID NO	Reverse Primer Start	Reverse Primer Stop	Reverse Primer Tm	Reverse Primer %GC	Primer Tm Diff	Product Length	Product Tm	Product %GC	Anneal Score	Optimum Anneal Temp
1	1021	12726	12746	51.3	47.6	3521	12996	12977	50.2	40	1	271	75	42.8	26	52.6
2	1022	12236	12256	51.2	42.9	3522	12993	12975	51.4	47.4	0.2	758	76.4	42.5	26	54
3	1023	12373	12391	50.8	47.4	3523	12993	12975	51.4	47.4	0.6	621	76.4	43	26	53.8
4	1024	12236	12256	51.2	42.9	3524	12996	12977	50.2	40	0.9	761	76.4	42.3	26	53.6
5	1025	12373	12391	50.8	47.4	3525	12996	12977	50.2	40	0.5	624	76.4	42.8	26	53.6
6	1026	12726	12746	51.3	47.6	3526	12993	12975	51.4	47.4	0.1	268	75.1	43.3	26	53.1
7	1027	2671	2692	52.1	40.9	3527	3185	3164	51	45.5	1.2	515	75.6	41.6	26	53.3
8	1028	28942	28961	50.2	45	3528	29298	29280	51.4	52.6	1.2	357	76.4	44.8	26	53.6
9	1029	19801	19819	53.2	52.6	3529	19922	19901	51.5	45.5	1.7	122	72.2	43.4	26	51.1
10	1030	19800	19817	50.4	50	3530	19921	19901	50.2	47.6	0.3	122	72.2	43.4	26	50.7
11	1031	9930	9948	51.5	52.6	3531	10605	10588	51.1	50	0.4	676	75.8	41.3	27	53.5
12	1032	9933	9952	50.9	45	3532	10605	10588	51.1	50	0.2	673	75.8	41.2	27	53.4
13	1033	9930	9949	52.2	50	3533	10605	10588	51.1	50	1.1	676	75.8	41.3	27	53.5

14	1034	9927	9945	50.8	52.6	3534	10605	10588	51.1	50	0.3	679	75.8	41.2	28	53.4
15	1035	3789	3806	50	50	3535	4445	4425	50.6	42.9	0.5	657	75.5	40.5	28	52.9
16	1036	3788	3805	50	50	3536	4444	4424	50.6	42.9	0.5	657	75.5	40.5	28	52.9
17	1037	3795	3813	52.1	52.6	3537	4445	4425	50.6	42.9	1.5	651	75.5	40.6	28	53.1
18	1038	3787	3804	50	50	3538	4445	4425	50.6	42.9	0.5	659	75.4	40.4	28	52.9
19	1039	19801	19819	53.2	52.6	3539	19921	19900	51.8	45.5	1.4	121	72.3	43.8	28	51.2
20	1040	24418	24436	50	47.4	3540	25182	25164	51.4	47.4	1.4	765	76.1	41.7	28	53.4
21	1041	9929	9949	53.8	47.6	3541	10449	10425	54.6	40	0.8	521	75.4	40.9	28	54
22	1042	2671	2692	52.1	40.9	3542	3186	3165	50.4	40.9	1.7	516	75.6	41.5	28	53.1
23	1043	3792	3810	52.9	52.6	3543	4446	4425	51.8	45.5	1.1	655	75.5	40.6	28	53.5
24	1044	9933	9952	50.9	45	3544	10449	10431	50.9	47.4	0.1	517	75.3	40.8	28	53.1
25	1045	3792	3810	52.9	52.6	3545	4445	4424	51.3	40.9	1.6	654	75.5	40.5	28	53.3
26	1046	25782	25806	53.5	40	3546	26184	26164	52.4	42.9	1.1	403	74.7	40.2	28	53.1
27	1047	9927	9945	50.8	52.6	3547	10449	10431	50.9	47.4	0.1	523	75.4	40.9	28	53.1
28	1048	9927	9945	50.8	52.6	3548	10449	10428	51.9	40.9	1.1	523	75.4	40.9	28	53.1
29	1049	3789	3806	50	50	3549	4444	4424	50.6	42.9	0.5	656	75.5	40.5	28	53
30	1050	3795	3813	52.1	52.6	3550	4444	4424	50.6	42.9	1.5	650	75.5	40.6	28	53.1
31	1051	9933	9952	50.9	45	3551	10449	10428	51.9	40.9	1.1	517	75.3	40.8	28	53.1
32	1052	9930	9948	51.5	52.6	3552	10449	10431	50.9	47.4	0.5	520	75.4	41	28	53.2
33	1053	9930	9948	51.5	52.6	3553	10449	10428	51.9	40.9	0.4	520	75.4	41	28	53.3
34	1054	9929	9948	53.2	50	3554	10449	10425	54.6	40	1.4	521	75.4	40.9	28	53.8
35	1055	9931	9952	53	45.5	3555	10449	10425	54.6	40	1.6	519	75.3	40.8	28	53.7
36	1056	3791	3808	50	50	3556	4445	4425	50.6	42.9	0.5	655	75.5	40.5	28	52.9
37	1057	3791	3808	50	50	3557	4444	4424	50.6	42.9	0.5	654	75.5	40.5	28	53
38	1058	9930	9949	52.2	50	3558	10449	10431	50.9	47.4	1.2	520	75.4	41	28	53.2
39	1059	9930	9949	52.2	50	3559	10449	10428	51.9	40.9	0.3	520	75.4	41	28	53.5
40	1060	3788	3805	50	50	3560	4445	4425	50.6	42.9	0.5	658	75.5	40.4	28	52.9
41	1061	19800	19817	50.4	50	3561	19921	19900	51.8	45.5	1.4	122	72.2	43.4	28	50.8
42	1062	3787	3804	50	50	3562	4444	4424	50.6	42.9	0.5	658	75.5	40.4	28	52.9
43	1063	25782	25806	53.5	40	3563	26183	26163	51.7	42.9	1.7	402	74.7	40.3	28	52.9
44	1064	25782	25806	53.5	40	3564	26183	26160	54.5	41.7	1	402	74.7	40.3	28	53.5
45	1065	25782	25806	53.5	40	3565	26183	26159	54.9	40	1.5	402	74.7	40.3	28	53.5
46	1066	2429	2447	50.2	47.4	3566	3187	3166	50.3	45.5	0.1	759	76.6	43	29	53.8
47	1067	2427	2445	52.1	52.6	3567	3185	3164	51	45.5	1.1	759	76.7	43.1	29	54.1
48	1068	2429	2447	50.2	47.4	3568	3185	3164	51	45.5	0.7	757	76.6	42.9	29	53.8
49	1069	19800	19817	50.4	50	3569	19923	19904	50.1	50	0.3	124	72.3	43.5	29	50.8
50	1070	2427	2445	52.1	52.6	3570	3187	3166	50.3	45.5	1.8	761	76.7	43.1	29	53.9
51	1071	29183	29204	50.4	40.9	3571	29412	29393	50.3	45	0	230	75.3	44.8	29	52.9
52	1072	16367	16386	51.4	50	3572	16780	16760	51.4	42.9	0.1	414	75	40.8	30	53
53	1073	11543	11562	50.4	40	3573	12254	12236	50.5	47.4	0.1	712	76.2	42	30	53.6
54	1074	12976	12995	51.1	45	3574	13547	13528	50.2	45	0.9	572	77.4	45.5	30	54.3
55	1075	12040	12057	50.6	50	3575	12254	12236	50.5	47.4	0.1	215	75.5	45.6	30	53.1
56	1076	12976	12996	51.8	42.9	3576	13544	13525	52.6	55	0.8	569	77.5	45.7	30	54.8
57	1077	10141	10160	51	45	3577	10605	10588	51.1	50	0.1	465	74.9	40.2	30	52.8
58	1078	12235	12253	50.1	52.6	3578	12996	12977	50.2	40	0.1	762	76.4	42.4	30	53.6
59	1079	19795	19814	50.4	45	3579	19921	19901	50.2	47.6	0.3	127	72.3	43.3	30	50.8
60	1080	12235	12253	50.1	52.6	3580	12993	12975	51.4	47.4	1.3	759	76.5	42.6	30	53.7
61	1081	12976	12994	50.3	47.4	3581	13547	13528	50.2	45	0.1	572	77.4	45.5	30	54.3
62	1082	12975	12994	52.1	45	3582	13544	13525	52.6	55	0.5	570	77.4	45.6	30	54.9
63	1083	12977	12996	50.2	40	3583	13547	13528	50.2	45	0	571	77.3	45.4	30	54.3
64	1084	11541	11561	50.9	42.9	3584	12254	12236	50.5	47.4	0.3	714	76.2	42	30	53.6
65	1085	28394	28411	50.3	50	3585	28672	28654	50.6	52.6	0.3	279	78.6	51.6	30	55.2

66	1086	9930	9948	51.5	52.6	3586	10455	10434	51.1	40.9	0.3	526	75.3	40.7	30	53.1
67	1087	8220	8238	51.5	47.4	3587	8929	8911	53.4	52.6	1.9	710	75.4	40	30	53.3
68	1088	9930	9949	52.2	50	3588	10455	10435	50.5	42.9	1.7	526	75.3	40.7	30	52.9
69	1089	12236	12256	51.2	42.9	3589	12412	12392	50	42.9	1.2	177	73	41.2	30	51.2
70	1090	9930	9949	52.2	50	3590	10455	10434	51.1	40.9	1.1	526	75.3	40.7	30	53.1
71	1091	9933	9952	50.9	45	3591	10455	10435	50.5	42.9	0.4	523	75.2	40.5	30	52.9
72	1092	12726	12746	51.3	47.6	3592	13314	13297	51	50	0.3	589	76.6	43.6	30	54
73	1093	9933	9952	50.9	45	3593	10455	10434	51.1	40.9	0.3	523	75.2	40.5	30	53
74	1094	16909	16928	50.8	45	3594	17501	17481	51.2	42.9	0.4	593	75.9	41.8	30	53.5
75	1095	12975	12993	51.4	47.4	3595	13544	13525	52.6	55	1.2	570	77.4	45.6	30	54.7
76	1096	2671	2692	52.1	40.9	3596	3187	3166	50.3	45.5	1.8	517	75.6	41.6	30	53.1
77	1097	19800	19818	52.1	52.6	3597	19921	19900	51.8	45.5	0.3	122	72.2	43.4	30	51.2
78	1098	12975	12993	51.4	47.4	3598	13547	13528	50.2	45	1.2	573	77.3	45.4	30	54.3
79	1099	9930	9948	51.5	52.6	3599	10455	10435	50.5	42.9	1	526	75.3	40.7	30	52.9
80	1100	12976	12995	51.1	45	3600	13544	13525	52.6	55	1.5	569	77.5	45.7	30	54.6
81	1101	24635	24653	50.5	52.6	3601	25182	25164	51.4	47.4	0.9	548	75.1	40.1	30	52.8
82	1102	24633	24651	50.1	52.6	3602	25182	25164	51.4	47.4	1.3	550	75.2	40.2	30	52.7
83	1103	24630	24648	50.8	52.6	3603	25182	25164	51.4	47.4	0.6	553	75.2	40.3	30	53
84	1104	28394	28412	51.1	47.4	3604	28672	28654	50.6	52.6	0.5	279	78.6	51.6	30	55.3
85	1105	28395	28413	50.2	42.1	3605	28672	28654	50.6	52.6	0.4	278	78.6	51.4	30	55.2
86	1106	28396	28415	51.2	45	3606	28672	28654	50.6	52.6	0.6	277	78.6	51.6	30	55.3
87	1107	26421	26441	51.5	42.9	3607	26587	26568	52.7	45	1.2	167	72.3	40.1	30	51.2
88	1108	26421	26441	51.5	42.9	3608	26589	26571	51.7	47.4	0.2	169	72.4	40.2	30	51.2
89	1109	26421	26441	51.5	42.9	3609	26589	26572	51	50	0.5	169	72.4	40.2	30	51.1
90	1110	26421	26441	51.5	42.9	3610	26590	26573	51.7	50	0.3	170	72.3	40	30	51.2
91	1111	26040	26061	56.4	54.5	3611	26589	26568	55.2	45.5	1.2	550	75.1	40	30	54.2
92	1112	26039	26057	52.6	52.6	3612	26183	26160	54.5	41.7	1.9	145	71.9	40.7	30	51.2
93	1113	26039	26057	52.6	52.6	3613	26182	26161	51.2	40.9	1.4	144	71.7	40.3	30	50.7
94	1114	26039	26057	52.6	52.6	3614	26183	26163	51.7	42.9	0.9	145	71.9	40.7	30	51
95	1115	8867	8887	52.3	47.6	3615	9253	9235	51.6	47.4	0.7	387	75.1	41.3	30	53.2
96	1116	10247	10267	50.5	47.6	3616	10605	10588	51.1	50	0.6	359	74.6	40.4	30	52.4
97	1117	11540	11557	50.4	50	3617	12254	12236	50.5	47.4	0.1	715	76.2	42.1	30	53.6
98	1118	11541	11560	50.1	45	3618	12254	12236	50.5	47.4	0.4	714	76.2	42	30	53.5
99	1119	8221	8240	52.4	50	3619	8929	8911	53.4	52.6	1	709	75.4	40.1	30	53.6
100	1120	13039	13057	51.1	52.6	3620	13177	13156	50.4	40.9	0.7	139	73.9	46	31	52
101	1121	19801	19819	53.2	52.6	3621	19917	19895	52.5	43.5	0.8	117	72	43.6	31	51.2
102	1122	19709	19730	51.3	40.9	3622	19921	19900	51.8	45.5	0.5	213	73.9	41.8	31	52.2
103	1123	16366	16386	54.4	52.4	3623	16774	16751	53.6	41.7	0.8	409	75.1	41.1	31	53.8
104	1124	3	21	53.4	52.6	3624	256	235	52.6	45.5	0.8	254	76.1	46.1	31	54.2
105	1125	4	22	52.3	52.6	3625	314	296	50.6	47.4	1.7	311	76.8	46.6	31	54.1
106	1126	13039	13058	51.8	50	3626	13177	13156	50.4	40.9	1.5	139	73.9	46	31	52
107	1127	19800	19817	50.4	50	3627	19916	19895	50.2	40.9	0.2	117	71.7	42.7	31	50.3
108	1128	4645	4665	50.2	42.9	3628	5306	5289	50.8	50	0.5	662	75.6	40.8	31	53.1
109	1129	13039	13057	51.1	52.6	3629	13747	13726	50.8	40.9	0.4	709	76.6	43.2	31	54
110	1130	13039	13058	51.8	50	3630	13747	13726	50.8	40.9	1.1	709	76.6	43.2	31	54
111	1131	3	21	53.4	52.6	3631	253	233	51.8	47.6	1.6	251	76.2	46.2	31	54
112	1132	27365	27385	53.2	47.6	3632	27464	27444	53	42.9	0.2	100	70.8	43	31	50.6
113	1133	24418	24436	50	47.4	3633	24527	24508	50.5	45	0.5	110	71.3	42.7	31	50
114	1134	26708	26727	50	45	3634	27463	27446	50	44.4	0	756	75.9	41.1	31	53.2
115	1135	24179	24200	53.3	40.9	3635	24936	24919	51.8	50	1.5	758	75.8	41	31	53.7
116	1136	26708	26727	50	45	3636	27462	27444	50.1	42.1	0.1	755	75.9	41.2	31	53.2

117	1137	26708	26731	54.2	41.7	3637	27465	27446	54.6	50	0.4	758	75.9	41.3	31	54.5
118	1138	27365	27384	52.6	50	3638	27464	27446	51.7	47.4	0.9	100	70.8	43	31	50.2
119	1139	27365	27384	52.6	50	3639	27464	27445	52.4	45	0.2	100	70.8	43	31	50.4
120	1140	27365	27384	52.6	50	3640	27464	27444	53	42.9	0.4	100	70.8	43	31	50.4
121	1141	27367	27385	51.4	52.6	3641	27571	27552	50.1	40	1.3	205	74.6	43.9	31	52.4
122	1142	27367	27385	51.4	52.6	3642	27567	27547	51.1	42.9	0.2	201	74.7	44.3	31	52.7
123	1143	2427	2445	52.1	52.6	3643	3186	3165	50.4	40.9	1.7	760	76.7	43	31	53.9
124	1144	8867	8887	52.3	47.6	3644	9256	9237	50.8	45	1.5	390	75.1	41.3	31	52.9
125	1145	9934	9953	50.7	50	3645	10605	10588	51.1	50	0.4	672	75.8	41.2	31	53.4
126	1146	2429	2447	50.2	47.4	3646	3186	3165	50.4	40.9	0.2	758	76.6	42.9	31	53.8
127	1147	27365	27385	53.2	47.6	3647	27464	27445	52.4	45	0.8	100	70.8	43	31	50.4
128	1148	19994	20011	50.4	50	3648	20615	20597	50.6	47.4	0.2	622	75.2	40	31	52.9
129	1149	9922	9941	51.3	50	3649	10605	10588	51.1	50	0.2	684	75.8	41.2	32	53.5
130	1150	12962	12980	50.7	47.4	3650	13544	13525	52.6	55	1.8	583	77.5	45.6	32	54.5
131	1151	12965	12988	54	41.7	3651	13544	13525	52.6	55	1.5	580	77.4	45.5	32	55
132	1152	13176	13197	52.7	45.5	3652	13544	13525	52.6	55	0.1	369	77.1	46.3	32	54.8
133	1153	28867	28886	53.2	50	3653	29298	29280	51.4	52.6	1.7	432	76.8	45.1	32	54.3
134	1154	24418	24439	52.9	45.5	3654	25182	25164	51.4	47.4	1.5	765	76.1	41.7	32	53.8
135	1155	24420	24440	50.8	42.9	3655	25182	25164	51.4	47.4	0.6	763	76.1	41.5	32	53.6
136	1156	8867	8887	52.3	47.6	3656	9107	9086	51.6	45.5	0.7	241	74.1	41.5	32	52.5
137	1157	1402	1422	50.2	42.9	3657	2103	2083	50.6	42.9	0.4	702	76.7	43.3	32	53.8
138	1158	25782	25805	52.1	41.7	3658	26183	26163	51.7	42.9	0.4	402	74.7	40.3	32	52.9
139	1159	25781	25805	53.5	40	3659	26183	26160	54.5	41.7	1	403	74.7	40.2	32	53.4
140	1160	25781	25805	53.5	40	3660	26183	26159	54.9	40	1.5	403	74.7	40.2	32	53.4
141	1161	2671	2692	52.1	40.9	3661	3052	3033	50.3	50	1.8	382	74.8	40.6	32	52.5
142	1162	12726	12746	51.3	47.6	3662	13177	13156	50.4	40.9	0.9	452	76.4	43.8	32	53.7
143	1163	16909	16928	50.8	45	3663	17111	17090	51.1	40.9	0.3	203	75	44.8	32	52.8
144	1164	12234	12252	50.6	47.4	3664	12993	12975	51.4	47.4	0.8	760	76.4	42.5	32	53.8
145	1165	26039	26057	52.6	52.6	3665	26828	26810	52.9	52.6	0.2	790	76.4	42.4	32	54.4
146	1166	26039	26057	52.6	52.6	3666	26694	26677	51.4	50	1.2	656	75.7	41	32	53.5
147	1167	26039	26057	52.6	52.6	3667	26692	26674	51.9	52.6	0.7	654	75.7	41	32	53.6
148	1168	26039	26057	52.6	52.6	3668	26691	26673	51.3	47.4	1.3	653	75.6	40.9	32	53.4
149	1169	26039	26057	52.6	52.6	3669	26687	26669	51.3	47.4	1.3	649	75.6	40.8	32	53.4
150	1170	26039	26057	52.6	52.6	3670	26684	26666	53.4	52.6	0.8	646	75.6	40.9	32	53.8
151	1171	26039	26057	52.6	52.6	3671	26683	26665	52.7	52.6	0.1	645	75.6	40.9	32	53.8
152	1172	9934	9953	50.7	50	3672	10449	10431	50.9	47.4	0.2	516	75.4	40.9	32	53.1
153	1173	9927	9945	50.8	52.6	3673	10455	10434	51.1	40.9	0.3	529	75.3	40.6	32	53
154	1174	7728	7746	51.7	52.6	3674	8188	8169	50.5	45	1.2	461	75.6	41.9	32	53.2
155	1175	18550	18571	50.4	40.9	3675	19216	19195	50.2	40.9	0.2	667	75.7	41.1	32	53.2
156	1176	19801	19819	53.2	52.6	3676	19921	19899	52.4	43.5	0.8	121	72.3	43.8	32	51.4
157	1177	19709	19730	51.3	40.9	3677	19923	19904	50.1	50	1.2	215	73.9	41.9	32	51.9
158	1178	4639	4659	51.1	47.6	3678	5306	5289	50.8	50	0.3	668	75.6	40.9	32	53.3
159	1179	19794	19813	50	50	3679	19921	19901	50.2	47.6	0.2	128	72.6	43.8	32	50.9
160	1180	12965	12985	51.2	42.9	3680	13544	13525	52.6	55	1.4	580	77.4	45.5	32	54.6
161	1181	9932	9953	53	45.5	3681	10449	10425	54.6	40	1.6	518	75.3	40.7	32	53.7
162	1182	19795	19814	50.4	45	3682	19921	19900	51.8	45.5	1.4	127	72.3	43.3	32	50.9
163	1183	27366	27384	52.2	52.6	3683	27468	27451	51.1	50	1	103	71.3	43.7	32	50.3
164	1184	27366	27384	52.2	52.6	3684	27467	27450	52.1	50	0.1	102	71.4	44.1	32	50.7
165	1185	27366	27384	52.2	52.6	3685	27466	27449	51	50	1.2	101	71.5	44.6	32	50.4
166	1186	25782	25805	52.1	41.7	3686	26183	26164	51	45	1.1	402	74.7	40.3	32	52.7
167	1187	9934	9953	50.7	50	3687	10449	10428	51.9	40.9	1.2	516	75.4	40.9	32	53.1
168	1188	9925	9945	53.4	52.4	3688	10449	10425	54.6	40	1.2	525	75.4	41	32	53.9

169	1189	19800	19817	50.4	50	3689	19922	19902	50	42.9	0.4	123	72.1	43.1	32	50.6
170	1190	8867	8887	52.3	47.6	3690	9310	9291	51.2	45	1.2	444	75.4	41.4	32	53.2
171	1191	27367	27385	51.4	52.6	3691	27468	27451	51.1	50	0.3	102	71.4	44.1	32	50.4
172	1192	27367	27385	51.4	52.6	3692	27467	27450	52.1	50	0.7	101	71.5	44.6	32	50.6
173	1193	2671	2692	52.1	40.9	3693	3082	3058	52.3	40	0.2	412	74.9	40.5	32	53.2
174	1194	9927	9945	50.8	52.6	3694	10608	10589	51	50	0.2	682	75.8	41.2	32	53.4
175	1195	19800	19817	50.4	50	3695	19920	19899	50.2	40.9	0.3	121	71.9	43	32	50.5
176	1196	13177	13197	50.3	42.9	3696	13547	13528	50.2	45	0.1	371	76.9	45.8	32	54
177	1197	28179	28200	50.8	40.9	3697	28672	28654	50.6	52.6	0.3	494	79.8	51.8	32	56.1
178	1198	27367	27385	51.4	52.6	3698	27466	27449	51	50	0.4	100	71.6	45	32	50.5
179	1199	27366	27385	52.8	50	3699	27465	27446	54.6	50	1.7	100	71.2	44	32	50.8
180	1200	19800	19818	52.1	52.6	3700	19921	19901	50.2	47.6	2	122	72.2	43.4	32	50.7
181	1201	9927	9945	50.8	52.6	3701	10455	10435	50.5	42.9	0.3	529	75.3	40.6	32	52.9
182	1202	28868	28887	50.7	45	3702	29298	29280	51.4	52.6	0.7	431	76.8	45	32	54.1
183	1203	28867	28887	53.7	47.6	3703	29306	29288	53.5	52.6	0.3	440	76.9	45.2	32	55
184	1204	28867	28887	53.7	47.6	3704	29301	29282	55.3	55	1.5	435	76.9	45.3	32	55.1
185	1205	28868	28888	51.4	42.9	3705	29298	29280	51.4	52.6	0	431	76.8	45	32	54.3
186	1206	28867	28888	54.3	45.5	3706	29306	29288	53.5	52.6	0.8	440	76.9	45.2	32	55
187	1207	28867	28888	54.3	45.5	3707	29301	29282	55.3	55	1	435	76.9	45.3	32	55.2
188	1208	28870	28889	50.1	40	3708	29298	29280	51.4	52.6	1.3	429	76.8	45	32	53.9
189	1209	28868	28889	52	40.9	3709	29306	29288	53.5	52.6	1.5	439	76.9	45.1	32	54.5
190	1210	28867	28889	54.8	43.5	3710	29301	29282	55.3	55	0.5	435	76.9	45.3	32	55.4
191	1211	28867	28890	55.2	41.7	3711	29306	29288	53.5	52.6	1.7	440	76.9	45.2	32	55
192	1212	28867	28890	55.2	41.7	3712	29301	29282	55.3	55	0.1	435	76.9	45.3	32	55.5
193	1213	28867	28890	55.2	41.7	3713	29299	29280	53.9	55	1.3	433	76.9	45.3	32	55.1
194	1214	12234	12252	50.6	47.4	3714	12996	12977	50.2	40	0.3	763	76.4	42.3	32	53.6
195	1215	28968	28988	50.9	47.6	3715	29298	29280	51.4	52.6	0.6	331	76.2	44.7	32	53.7
196	1216	28968	28989	51.5	45.5	3716	29298	29280	51.4	52.6	0.1	331	76.2	44.7	32	53.9
197	1217	13230	13251	52.4	45.5	3717	13544	13525	52.6	55	0.1	315	77.2	47.3	32	54.8
198	1218	29186	29205	50.1	40	3718	29298	29280	51.4	52.6	1.3	113	72.8	46	32	51.1
199	1219	29195	29213	51.9	52.6	3719	29306	29288	53.5	52.6	1.6	112	73.6	48.2	32	52.2
200	1220	29195	29213	51.9	52.6	3720	29298	29280	51.4	52.6	0.5	104	73.1	48.1	32	51.7
201	1221	29196	29214	51.1	52.6	3721	29298	29280	51.4	52.6	0.3	103	73.3	48.5	32	51.7
202	1222	29195	29214	52.6	50	3722	29306	29288	53.5	52.6	0.9	112	73.6	48.2	32	52.4
203	1223	29196	29215	51.8	50	3723	29306	29288	53.5	52.6	1.6	111	73.8	48.6	32	52.3
204	1224	29196	29215	51.8	50	3724	29298	29280	51.4	52.6	0.4	103	73.3	48.5	32	51.8
205	1225	29197	29216	50	45	3725	29298	29280	51.4	52.6	1.4	102	73	48	32	51.2
206	1226	29196	29216	52.5	47.6	3726	29306	29288	53.5	52.6	1	111	73.8	48.6	32	52.5
207	1227	29195	29216	53.8	45.5	3727	29301	29282	55.3	55	1.5	107	73.5	48.6	32	52.7
208	1228	29254	29273	53.1	50	3728	29358	29339	52.8	50	0.2	105	73.4	48.6	32	52.3
209	1229	29259	29278	52.6	50	3729	29358	29339	52.8	50	0.2	100	72.4	47	32	51.6
210	1230	1402	1422	50.2	42.9	3730	1773	1755	51.7	52.6	1.5	372	75.8	43.3	33	53.2
211	1231	12726	12746	51.3	47.6	3731	13326	13306	50.7	42.9	0.6	601	76.7	43.6	33	54
212	1232	4	22	52.3	52.6	3732	269	251	51.1	52.6	1.2	266	76.5	46.6	33	54
213	1233	19800	19817	50.4	50	3733	19923	19903	50.9	47.6	0.4	124	72.3	43.5	33	50.9
214	1234	2371	2389	50.3	47.4	3734	3082	3058	52.3	40	2	712	76.7	43.3	33	53.9
215	1235	3	21	53.4	52.6	3735	270	251	52.9	50	0.5	268	76.4	46.3	33	54.4
216	1236	9930	9949	52.2	50	3736	10183	10166	50.9	50	1.3	254	75.3	44.1	33	53.1
217	1237	19795	19814	50.4	45	3737	19923	19904	50.1	50	0.3	129	72.5	43.4	33	50.9
218	1238	8867	8887	52.3	47.6	3738	9365	9347	53	52.6	0.7	499	75.8	42.1	33	53.9
219	1239	2371	2389	50.3	47.4	3739	3055	3036	50.6	50	0.3	685	76.7	43.4	33	53.9

220	1240	19709	19730	51.3	40.9	3740	19921	19901	50.2	47.6	1.1	213	73.9	41.8	33	51.9
221	1241	9930	9949	52.2	50	3741	10183	10165	51.7	47.4	0.5	254	75.3	44.1	33	53.3
222	1242	2371	2389	50.3	47.4	3742	2747	2727	50	42.9	0.3	377	76.9	45.9	33	54
223	1243	24921	24938	50.4	50	3743	25182	25164	51.4	47.4	1	262	74.2	41.2	33	52.2
224	1244	18077	18099	54.4	47.8	3744	18443	18424	55.9	55	1.5	367	75.8	43.3	33	54.5
225	1245	25772	25793	52.4	40.9	3745	26183	26164	51	45	1.3	412	74.8	40.3	33	52.8
226	1246	25769	25786	50.3	50	3746	26183	26164	51	45	0.8	415	74.9	40.5	33	52.6
227	1247	25348	25366	51.2	47.4	3747	25548	25531	51.1	50	0.1	201	74.3	43.3	33	52.4
228	1248	12726	12746	51.3	47.6	3748	13323	13304	51.1	45	0.2	598	76.7	43.6	33	54.1
229	1249	8372	8390	50.7	47.4	3749	8928	8911	51.9	50	1.2	557	75.1	40	33	52.9
230	1250	2671	2692	52.1	40.9	3750	3189	3168	51	45.5	1.2	519	75.7	41.6	33	53.4
231	1251	25348	25365	50.4	50	3751	25548	25531	51.1	50	0.7	201	74.3	43.3	33	52.2
232	1252	19801	19819	53.2	52.6	3752	19923	19902	51.5	45.5	1.7	123	72.4	43.9	33	51.3
233	1253	27442	27461	51.5	40	3753	27546	27527	51.3	50	0.2	105	71.8	44.8	33	50.8
234	1254	8867	8887	52.3	47.6	3754	9312	9293	50.6	45	1.8	446	75.4	41.5	33	53
235	1255	2671	2692	52.1	40.9	3755	3056	3038	50.8	52.6	1.3	386	74.8	40.7	33	52.7
236	1256	13231	13251	50.1	42.9	3756	13547	13528	50.2	45	0.2	317	76.9	46.7	33	54
237	1257	9055	9079	52.8	40	3757	9310	9291	51.2	45	1.7	256	74.4	41.8	33	52.5
238	1258	28821	28838	50.3	50	3758	29298	29280	51.4	52.6	1.1	478	77	45.2	33	54.1
239	1259	9055	9079	52.8	40	3759	9253	9235	51.6	47.4	1.2	199	73.6	41.7	33	52.1
240	1260	23840	23863	55.2	45.8	3760	24050	24031	56.5	55	1.4	211	75	44.5	33	54.1
241	1261	18074	18093	50.3	45	3761	18233	18214	52	50	1.7	160	73.9	44.4	33	51.9
242	1262	27366	27384	52.2	52.6	3762	27674	27654	51.9	42.9	0.3	309	74.3	40.5	33	52.7
243	1263	28967	28989	53.7	47.8	3763	29301	29282	55.3	55	1.5	335	76.4	45.1	33	54.7
244	1264	27366	27384	52.2	52.6	3764	27674	27653	52.5	40.9	0.3	309	74.3	40.5	33	52.8
245	1265	28966	28988	55.3	52.2	3765	29301	29282	55.3	55	0.1	336	76.4	45.2	33	55.2
246	1266	18074	18094	51.1	42.9	3766	18233	18214	52	50	1	160	73.9	44.4	33	52.1
247	1267	28965	28984	52.9	55	3767	29298	29280	51.4	52.6	1.5	334	76.4	45.2	33	54
248	1268	18081	18099	51.2	52.6	3768	18233	18215	51.3	52.6	0.1	153	74	45.1	33	52.2
249	1269	18081	18099	51.2	52.6	3769	18233	18214	52	50	0.8	153	74	45.1	33	52.2
250	1270	18081	18099	51.2	52.6	3770	18231	18210	52.2	45.5	1	151	73.6	44.4	33	52
251	1271	24480	24500	53.2	47.6	3771	24815	24791	54.5	40	1.3	336	75.6	43.2	33	54
252	1272	24481	24503	52.7	43.5	3772	24815	24791	54.5	40	1.8	335	75.5	43	33	53.8
253	1273	27367	27385	51.4	52.6	3773	27675	27656	50	40	1.4	309	74.3	40.5	33	52.1
254	1274	27367	27385	51.4	52.6	3774	27674	27654	51.9	42.9	0.5	308	74.4	40.6	33	52.6
255	1275	27367	27385	51.4	52.6	3775	27674	27653	52.5	40.9	1.1	308	74.4	40.6	33	52.6
256	1276	18081	18099	51.2	52.6	3776	18223	18206	51.8	50	0.6	143	73.2	44.1	33	51.7
257	1277	18080	18099	53	50	3777	18220	18202	54.8	52.6	1.9	141	73.1	44	33	52.2
258	1278	9933	9952	50.9	45	3778	10670	10649	51.3	40.9	0.5	738	75.7	40.8	33	53.4
259	1279	27665	27686	51.4	40.9	3779	28208	28190	51.7	52.6	0.4	544	75.1	40.1	33	53.1
260	1280	27665	27685	50.7	42.9	3780	28208	28190	51.7	52.6	1.1	544	75.1	40.1	33	52.9
261	1281	27442	27461	51.5	40	3781	27541	27522	50.1	45	1.4	100	71.2	44	33	50
262	1282	28821	28840	51.8	45	3782	29298	29280	51.4	52.6	0.4	478	77	45.2	33	54.4
263	1283	28821	28839	51.1	47.4	3783	29298	29280	51.4	52.6	0.3	478	77	45.2	33	54.3
264	1284	8868	8889	50.4	40.9	3784	9252	9235	50.1	50	0.3	385	75.1	41.3	34	52.7
265	1285	19800	19818	52.1	52.6	3785	19920	19899	50.2	40.9	2	121	71.9	43	34	50.5
266	1286	9055	9079	52.8	40	3786	9313	9293	52.1	47.6	0.7	259	74.6	42.1	34	52.9
267	1287	10142	10163	51.3	40.9	3787	10605	10588	51.1	50	0.2	464	74.9	40.1	34	52.8
268	1288	12726	12746	51.3	47.6	3788	13312	13294	51	52.6	0.3	587	76.6	43.6	34	54
269	1289	9055	9079	52.8	40	3789	9257	9237	52.2	42.9	0.7	203	73.5	41.4	34	52.2
270	1290	7876	7895	51.5	45	3790	8188	8169	50.5	45	1.1	313	75	42.2	34	52.8

271	1291	23843	23863	50.3	42.9	3791	24527	24507	51	42.9	0.7	685	76	41.8	34	53.4
272	1292	10247	10267	50.5	47.6	3792	10608	10589	51	50	0.5	362	74.6	40.3	34	52.4
273	1293	24179	24199	52.7	42.9	3793	24815	24791	54.5	40	1.8	637	75.8	41.3	34	53.9
274	1294	12236	12256	51.2	42.9	3794	12998	12979	50.1	45	1.1	763	76.4	42.5	34	53.6
275	1295	7869	7889	52.5	47.6	3795	8189	8169	52	47.6	0.5	321	75.3	42.7	34	53.4
276	1296	1402	1422	50.2	42.9	3796	2152	2133	50.7	45	0.5	751	76.7	43.1	34	53.8
277	1297	12233	12251	51.1	52.6	3797	12993	12975	51.4	47.4	0.2	761	76.5	42.6	34	54
278	1298	3033	3053	51.7	47.6	3798	3650	3631	53.1	50	1.4	618	76.4	42.9	34	54.1
279	1299	12233	12251	51.1	52.6	3799	12996	12977	50.2	40	0.9	764	76.4	42.4	34	53.7
280	1300	24483	24503	51	42.9	3800	24938	24921	50.4	50	0.6	456	75.6	41.9	34	53.1
281	1301	11541	11561	50.9	42.9	3801	12253	12235	50.1	52.6	0.8	713	76.2	42.1	34	53.5
282	1302	24622	24643	57.1	54.5	3802	25400	25379	56	50	1.1	779	75.7	40.7	34	54.9
283	1303	24622	24643	57.1	54.5	3803	25400	25378	56.4	47.8	0.6	779	75.7	40.7	34	55
284	1304	24630	24648	50.8	52.6	3804	25403	25385	51.1	47.4	0.3	774	75.7	40.6	34	53.3
285	1305	9929	9946	50	50	3805	10605	10588	51.1	50	1	677	75.8	41.2	34	53.2
286	1306	24633	24651	50.1	52.6	3806	25403	25385	51.1	47.4	1	771	75.6	40.5	34	53.1
287	1307	11541	11560	50.1	45	3807	12253	12235	50.1	52.6	0	713	76.2	42.1	34	53.5
288	1308	24635	24653	50.5	52.6	3808	25403	25385	51.1	47.4	0.7	769	75.6	40.4	34	53.2
289	1309	9933	9952	50.9	45	3809	10608	10589	51	50	0.1	676	75.8	41.1	34	53.4
290	1310	24921	24938	50.4	50	3810	25548	25531	51.1	50	0.7	628	75.6	40.9	34	53.1
291	1311	7725	7743	50.8	47.4	3811	8188	8169	50.5	45	0.4	464	75.6	41.8	34	53.2
292	1312	28547	28568	53.5	45.5	3812	29301	29282	55.3	55	1.8	755	78.5	47.5	34	56.1
293	1313	28547	28568	53.5	45.5	3813	29306	29288	53.5	52.6	0	760	78.5	47.5	34	56.1
294	1314	28548	28568	50.5	42.9	3814	29298	29280	51.4	52.6	0.9	751	78.4	47.4	34	55.2
295	1315	28546	28567	55.1	50	3815	29301	29282	55.3	55	0.2	756	78.5	47.6	34	56.6
296	1316	28547	28567	52.9	47.6	3816	29298	29280	51.4	52.6	1.5	752	78.5	47.5	34	55.5
297	1317	28546	28565	52.2	50	3817	29298	29280	51.4	52.6	0.8	753	78.5	47.5	34	55.5
298	1318	28546	28565	52.2	50	3818	29306	29288	53.5	52.6	1.3	761	78.5	47.6	34	55.7
299	1319	28396	28416	52.4	47.6	3819	28672	28654	50.6	52.6	1.8	277	78.6	51.6	34	55.3
300	1320	28396	28415	51.2	45	3820	28671	28652	52.8	55	1.6	276	78.6	51.4	34	55.4
301	1321	28396	28415	51.2	45	3821	28671	28653	50.2	52.6	1	276	78.6	51.4	34	55.2
302	1322	12976	12995	51.1	45	3822	13545	13527	50.3	52.6	0.8	570	77.4	45.6	34	54.4
303	1323	16551	16568	51.1	50	3823	16711	16691	51	42.9	0.1	161	73.8	44.1	34	52.1
304	1324	28395	28414	51.5	45	3824	28672	28654	50.6	52.6	0.9	278	78.6	51.4	34	55.3
305	1325	16555	16572	50.3	50	3825	16780	16760	51.4	42.9	1.1	226	73.6	40.7	34	51.7
306	1326	28394	28413	51.8	45	3826	28671	28652	52.8	55	1	278	78.6	51.4	34	55.6
307	1327	28395	28413	50.2	42.1	3827	28671	28653	50.2	52.6	0	277	78.5	51.3	34	55.1
308	1328	7728	7746	51.7	52.6	3828	8049	8032	50.4	50	1.3	322	74.9	41.6	34	52.6
309	1329	28394	28412	51.1	47.4	3829	28671	28652	52.8	55	1.7	278	78.6	51.4	34	55.4
310	1330	28394	28412	51.1	47.4	3830	28671	28653	50.2	52.6	0.8	278	78.6	51.4	34	55.2
311	1331	11543	11562	50.4	40	3831	12257	12237	51.3	47.6	0.9	715	76.2	42	34	53.5
312	1332	28393	28411	52.9	52.6	3832	28671	28652	52.8	55	0.1	279	78.6	51.6	34	56
313	1333	28394	28411	50.3	50	3833	28671	28653	50.2	52.6	0	278	78.6	51.4	34	55.2
314	1334	4255	4276	51.7	45.5	3834	4710	4691	50.2	45	1.5	456	75.1	40.8	34	52.8
315	1335	12975	12994	52.1	45	3835	13545	13526	52.9	55	0.8	571	77.4	45.5	34	54.9
316	1336	9930	9948	51.5	52.6	3836	10608	10589	51	50	0.5	679	75.8	41.2	34	53.5
317	1337	27665	27686	51.4	40.9	3837	28411	28393	52.9	52.6	1.6	747	76.8	43.5	34	54.3
318	1338	27665	27686	51.4	40.9	3838	28415	28396	51.2	45	0.2	751	76.8	43.4	34	54.2
319	1339	11541	11561	50.9	42.9	3839	12257	12237	51.3	47.6	0.5	717	76.2	42	34	53.7
320	1340	27665	27685	50.7	42.9	3840	28415	28396	51.2	45	0.5	751	76.8	43.4	34	54.1
321	1341	11543	11562	50.4	40	3841	12253	12235	50.1	52.6	0.3	711	76.2	42.1	34	53.5

322	1342	11545	11563	50.8	47.4	3842	12254	12236	50.5	47.4	0.2	710	76.2	42.1	34	53.6
323	1343	27436	27455	52.7	45	3843	27542	27522	50.9	42.9	1.8	107	72	44.9	34	50.8
324	1344	27436	27455	52.7	45	3844	27546	27527	51.3	50	1.3	111	72.7	45.9	34	51.4
325	1345	27389	27407	50.6	47.4	3845	27541	27522	50.1	45	0.5	153	73.2	43.1	34	51.4
326	1346	27389	27407	50.6	47.4	3846	27546	27527	51.3	50	0.7	158	73.5	43.7	34	51.8
327	1347	27369	27392	57.2	50	3847	27468	27446	57.7	47.8	0.5	100	71.2	44	34	52.1
328	1348	27367	27389	55	47.8	3848	27466	27446	56.8	52.4	1.8	100	71.6	45	34	51.7
329	1349	11541	11560	50.1	45	3849	12257	12237	51.3	47.6	1.2	717	76.2	42	34	53.5
330	1350	7725	7742	50	50	3850	8188	8169	50.5	45	0.4	464	75.6	41.8	34	53
331	1351	2223	2243	50.2	42.9	3851	2672	2653	51.6	50	1.4	450	77	45.3	34	54.1
332	1352	9930	9949	52.2	50	3852	10608	10589	51	50	1.2	679	75.8	41.2	34	53.5
333	1353	9934	9953	50.7	50	3853	10455	10435	50.5	42.9	0.3	522	75.3	40.6	34	52.9
334	1354	2223	2243	50.2	42.9	3854	2672	2654	50.9	52.6	0.7	450	77	45.3	34	54.1
335	1355	3797	3815	50.9	47.4	3855	4445	4425	50.6	42.9	0.4	649	75.4	40.4	34	53.1
336	1356	9934	9953	50.7	50	3856	10455	10434	51.1	40.9	0.4	522	75.3	40.6	34	53
337	1357	18074	18093	50.3	45	3857	18697	18679	51.9	52.6	1.5	624	76.2	42.5	34	53.6
338	1358	12976	12994	50.3	47.4	3858	13545	13527	50.3	52.6	0.1	570	77.4	45.6	34	54.4
339	1359	12040	12057	50.6	50	3859	12498	12480	50	47.4	0.6	459	76.3	43.6	34	53.5
340	1360	12040	12057	50.6	50	3860	12257	12237	51.3	47.6	0.7	218	75.4	45.4	34	53.1
341	1361	11540	11557	50.4	50	3861	12257	12237	51.3	47.6	0.9	718	76.2	42.1	34	53.6
342	1362	12975	12993	51.4	47.4	3862	13545	13526	52.9	55	1.5	571	77.4	45.5	34	54.7
343	1363	12975	12993	51.4	47.4	3863	13545	13527	50.3	52.6	1.1	571	77.4	45.5	34	54.4
344	1364	11540	11560	53.2	47.6	3864	11983	11965	53	52.6	0.2	444	75.1	40.8	34	53.6
345	1365	12040	12057	50.6	50	3865	12253	12235	50.1	52.6	0.5	214	75.5	45.8	34	53
346	1366	12976	12995	51.1	45	3866	13545	13526	52.9	55	1.8	570	77.4	45.6	34	54.6
347	1367	13039	13057	51.1	52.6	3867	13314	13297	51	50	0.1	276	75.7	44.6	34	53.4
348	1368	27361	27380	52.4	55	3868	27463	27444	51.6	40	0.8	103	71.7	44.7	34	50.7
349	1369	27361	27380	52.4	55	3869	27463	27445	50.8	42.1	1.6	103	71.7	44.7	34	50.5
350	1370	27361	27380	52.4	55	3870	27464	27446	51.7	47.4	0.7	104	71.9	45.2	34	51
351	1371	25348	25365	50.4	50	3871	25645	25626	50.8	45	0.4	298	74.6	41.3	34	52.4
352	1372	9922	9941	51.3	50	3872	10449	10431	50.9	47.4	0.3	528	75.4	40.9	34	53.2
353	1373	13039	13057	51.1	52.6	3873	13323	13304	51.1	45	0	285	75.8	44.6	34	53.5
354	1374	12235	12253	50.1	52.6	3874	12412	12392	50	42.9	0.1	178	73.2	41.6	34	51.3
355	1375	3016	3036	50.2	42.9	3875	3185	3164	51	45.5	0.7	170	74.5	45.3	34	52.3
356	1376	13039	13057	51.1	52.6	3876	13326	13306	50.7	42.9	0.4	288	75.8	44.4	34	53.4
357	1377	7869	7889	52.5	47.6	3877	8050	8032	52	52.6	0.5	182	73.8	42.9	34	52.3
358	1378	26421	26441	51.5	42.9	3878	26655	26634	50.6	40.9	0.9	235	74.1	41.7	34	52.2
359	1379	26421	26441	51.5	42.9	3879	26657	26639	50.8	47.4	0.7	237	74.2	41.8	34	52.3
360	1380	26040	26061	56.4	54.5	3880	26183	26159	54.9	40	1.5	144	72	41	34	52
361	1381	26040	26061	56.4	54.5	3881	26183	26160	54.5	41.7	2	144	72	41	34	51.9
362	1382	26040	26061	56.4	54.5	3882	26184	26161	55.1	41.7	1.3	145	71.9	40.7	34	52
363	1383	12373	12391	50.8	47.4	3883	12724	12705	52.4	55	1.6	352	75.6	42.9	34	53.2
364	1384	26040	26061	56.4	54.5	3884	26589	26569	54.7	47.6	1.7	550	75.1	40	34	54.1
365	1385	26039	26058	54	55	3885	26183	26159	54.9	40	0.9	145	71.9	40.7	34	51.7
366	1386	26039	26058	54	55	3886	26183	26160	54.5	41.7	0.4	145	71.9	40.7	34	51.7
367	1387	26039	26058	54	55	3887	26183	26161	54	43.5	0	145	71.9	40.7	34	51.7
368	1388	26039	26058	54	55	3888	26184	26163	53	40.9	1	146	71.8	40.4	34	51.3
369	1389	26039	26057	52.6	52.6	3889	26174	26153	51	40.9	1.6	136	71.8	41.2	34	50.7
370	1390	10246	10266	50.4	47.6	3890	10605	10588	51.1	50	0.6	360	74.5	40.3	34	52.4
371	1391	3234	3254	51.1	47.6	3891	3497	3478	51.3	50	0.2	264	74.3	41.3	34	52.4
372	1392	26039	26057	52.6	52.6	3892	26183	26162	52.8	45.5	0.2	145	71.9	40.7	34	51.2

373	1393	11540	11557	50.4	50	3893	12253	12235	50.1	52.6	0.3	714	76.2	42.2	34	53.5
374	1394	3234	3254	51.1	47.6	3894	3500	3481	51.2	50	0.1	267	74.3	41.2	34	52.4
375	1395	3794	3812	52.9	52.6	3895	4445	4424	51.3	40.9	1.6	652	75.5	40.5	34	53.3
376	1396	3794	3812	52.9	52.6	3896	4446	4425	51.8	45.5	1.1	653	75.5	40.6	34	53.5
377	1397	3234	3254	51.1	47.6	3897	3646	3625	52	40.9	1	413	75.1	41.2	34	53
378	1398	3234	3254	51.1	47.6	3898	3647	3628	50.6	45	0.5	414	75.2	41.3	34	52.9
379	1399	3226	3245	51.7	55	3899	3497	3478	51.3	50	0.4	272	74.6	41.9	34	52.7
380	1400	3797	3815	50.9	47.4	3900	4444	4424	50.6	42.9	0.4	648	75.4	40.4	34	53.1
381	1401	3226	3245	51.7	55	3901	3500	3481	51.2	50	0.5	275	74.6	41.8	34	52.7
382	1402	16366	16384	50.3	52.6	3902	16780	16760	51.4	42.9	1.1	415	75.1	41	34	52.7
383	1403	25782	25806	53.5	40	3903	26183	26161	54	43.5	0.5	402	74.7	40.3	34	53.5
384	1404	16366	16385	52.9	55	3904	16780	16760	51.4	42.9	1.4	415	75.1	41	34	53.1
385	1405	16367	16386	51.4	50	3905	16781	16761	51.3	47.6	0.1	415	75.1	41	34	53
386	1406	12236	12256	51.2	42.9	3906	12992	12974	51.2	52.6	0	757	76.5	42.5	34	54
387	1407	16367	16386	51.4	50	3907	16777	16758	51.5	50	0.1	411	75	40.9	34	53
388	1408	16367	16386	51.4	50	3908	16711	16691	51	42.9	0.3	345	75.2	42	34	53
389	1409	3226	3245	51.7	55	3909	3503	3484	51.5	50	0.3	278	74.7	42.1	34	52.9
390	1410	16548	16566	54.9	52.6	3910	16782	16760	54.3	43.5	0.6	235	74	41.3	34	53.2
391	1411	16549	16567	54.9	52.6	3911	16782	16760	54.3	43.5	0.6	234	74	41.5	34	53.2
392	1412	25354	25372	50.9	52.6	3912	25645	25626	50.8	45	0.2	292	74.4	41.1	34	52.4
393	1413	16551	16568	51.1	50	3913	17038	17021	50.7	50	0.4	488	75.8	42.2	34	53.4
394	1414	25348	25366	51.2	47.4	3914	25645	25626	50.8	45	0.4	298	74.6	41.3	34	52.5
395	1415	16551	16568	51.1	50	3915	16780	16760	51.4	42.9	0.3	230	73.9	41.3	34	52.2
396	1416	7725	7743	50.8	47.4	3916	8049	8032	50.4	50	0.5	325	74.9	41.5	34	52.6
397	1417	29200	29224	54.2	40	3917	29299	29280	53.9	55	0.3	100	72.8	48	34	52.3
398	1418	29200	29224	54.2	40	3918	29301	29282	55.3	55	1.1	102	73	48	34	52.4
399	1419	29200	29223	53.7	41.7	3919	29299	29280	53.9	55	0.2	100	72.8	48	34	52.2
400	1420	29200	29223	53.7	41.7	3920	29301	29282	55.3	55	1.6	102	73	48	34	52.3
401	1421	29199	29222	54.6	41.7	3921	29301	29282	55.3	55	0.7	103	72.9	47.6	34	52.5
402	1422	29200	29222	53.2	43.5	3922	29299	29280	53.9	55	0.7	100	72.8	48	34	52
403	1423	29199	29221	54.1	43.5	3923	29301	29282	55.3	55	1.2	103	72.9	47.6	34	52.3
404	1424	29200	29221	52.6	45.5	3924	29299	29280	53.9	55	1.3	100	72.8	48	34	51.9
405	1425	18074	18093	50.3	45	3925	18239	18220	50	45	0.3	166	73.9	44	34	51.8
406	1426	18074	18093	50.3	45	3926	18238	18219	50.3	45	0	165	74	44.2	34	51.9
407	1427	1402	1426	54.1	40	3927	1774	1755	53.1	50	1	373	75.8	43.2	34	54.1
408	1428	18074	18094	51.1	42.9	3928	18697	18679	51.9	52.6	0.8	624	76.2	42.5	34	53.8
409	1429	18074	18094	51.1	42.9	3929	18239	18220	50	45	1	166	73.9	44	34	51.8
410	1430	18074	18094	51.1	42.9	3930	18238	18219	50.3	45	0.8	165	74	44.2	34	51.9
411	1431	3226	3245	51.7	55	3931	3504	3485	50.4	45	1.3	279	74.7	41.9	34	52.5
412	1432	18081	18099	51.2	52.6	3932	18662	18641	50.4	40.9	0.7	582	76.3	42.8	34	53.6
413	1433	7725	7742	50	50	3933	8049	8032	50.4	50	0.3	325	74.9	41.5	34	52.5
414	1434	29182	29205	54.6	41.7	3934	29301	29282	55.3	55	0.7	120	73.4	46.7	34	52.9
415	1435	4255	4276	51.7	45.5	3935	4711	4692	51.2	45	0.5	457	75.1	40.7	34	53
416	1436	29183	29204	50.4	40.9	3936	29298	29280	51.4	52.6	1.1	116	72.8	45.7	34	51.2
417	1437	3225	3243	50.9	52.6	3937	3497	3478	51.3	50	0.4	273	74.7	42.1	34	52.7
418	1438	29181	29202	53.9	45.5	3938	29301	29282	55.3	55	1.4	121	73.6	47.1	34	52.8
419	1439	29182	29202	51.2	42.9	3939	29298	29280	51.4	52.6	0.2	117	73.1	46.2	34	51.6
420	1440	29180	29199	50.1	40	3940	29298	29280	51.4	52.6	1.3	119	73.2	46.2	34	51.4
421	1441	28970	28993	53.3	41.7	3941	29301	29282	55.3	55	1.9	332	76.1	44.6	34	54.4
422	1442	28971	28993	51.9	43.5	3942	29298	29280	51.4	52.6	0.5	328	76.1	44.5	34	53.8
423	1443	4255	4276	51.7	45.5	3943	4711	4693	50.4	47.4	1.3	457	75.1	40.7	34	52.8
424	1444	12976	12996	51.8	42.9	3944	13545	13526	52.9	55	1.1	570	77.4	45.6	34	54.8

425	1445	28968	28989	51.5	45.5	3945	29306	29288	53.5	52.6	2	339	76.3	44.8	34	54
426	1446	3225	3243	50.9	52.6	3946	3500	3481	51.2	50	0.3	276	74.7	42	34	52.6
427	1447	3225	3243	50.9	52.6	3947	3503	3484	51.5	50	0.6	279	74.8	42.3	34	52.7
428	1448	3225	3243	50.9	52.6	3948	3504	3485	50.4	45	0.5	280	74.8	42.1	34	52.6
429	1449	28939	28961	55.2	47.8	3949	29301	29282	55.3	55	0.1	363	76.6	45.2	34	55.3
430	1450	28941	28961	51.6	42.9	3950	29306	29288	53.5	52.6	1.8	366	76.4	44.8	34	54.1
431	1451	8867	8886	50.7	50	3951	9252	9235	50.1	50	0.6	386	75.1	41.5	34	52.7
432	1452	28939	28960	54.7	50	3952	29301	29282	55.3	55	0.6	363	76.6	45.2	34	55.1
433	1453	28940	28960	52.4	47.6	3953	29306	29288	53.5	52.6	1	367	76.5	45	34	54.4
434	1454	28941	28960	50.9	45	3954	29298	29280	51.4	52.6	0.5	358	76.3	44.7	34	53.8
435	1455	3360	3380	51.4	42.9	3955	3494	3473	50.4	40.9	1	135	73.7	45.9	34	51.8
436	1456	19709	19730	51.3	40.9	3956	19916	19895	50.2	40.9	1	208	73.6	41.3	34	51.7
437	1457	12373	12391	50.8	47.4	3957	12994	12976	50.3	47.4	0.4	622	76.4	42.9	34	53.7
438	1458	19794	19813	50	50	3958	19921	19900	51.8	45.5	1.8	128	72.6	43.8	34	50.9
439	1459	3361	3381	50.5	42.9	3959	3494	3473	50.4	40.9	0.1	134	73.8	46.3	34	51.9
440	1460	12234	12252	50.6	47.4	3960	12992	12974	51.2	52.6	0.6	759	76.5	42.6	34	53.8
441	1461	12234	12252	50.6	47.4	3961	12994	12976	50.3	47.4	0.2	761	76.4	42.4	34	53.7
442	1462	3034	3053	50.3	50	3962	3647	3628	50.6	45	0.3	614	76.4	42.8	34	53.6
443	1463	8867	8887	52.3	47.6	3963	9254	9236	50.6	47.4	1.7	388	75.1	41.2	34	52.8
444	1464	12726	12746	51.3	47.6	3964	12994	12976	50.3	47.4	1	269	75.1	43.1	34	52.8
445	1465	12234	12252	50.6	47.4	3965	12998	12979	50.1	45	0.5	765	76.4	42.5	34	53.6
446	1466	9926	9944	50.5	52.6	3966	10605	10588	51.1	50	0.6	680	75.8	41.2	34	53.3
447	1467	3034	3053	50.3	50	3967	3646	3625	52	40.9	1.7	613	76.3	42.7	34	53.6
448	1468	12977	12996	50.2	40	3968	13545	13527	50.3	52.6	0	569	77.4	45.5	34	54.3
449	1469	19799	19817	52.2	52.6	3969	19909	19885	52.5	40	0.3	111	71.6	43.2	34	50.8
450	1470	8867	8887	52.3	47.6	3970	9246	9226	50.5	42.9	1.8	380	75	41.1	34	52.7
451	1471	8867	8887	52.3	47.6	3971	9342	9323	52.1	50	0.3	476	75.7	42	35	53.7
452	1472	10141	10160	51	45	3972	10608	10589	51	50	0	468	74.9	40.2	35	52.8
453	1473	3192	3213	51.8	45.5	3973	3494	3473	50.4	40.9	1.4	303	74.9	41.9	35	52.6
454	1474	3360	3379	50.7	45	3974	3647	3628	50.6	45	0.1	288	75.5	43.8	35	53.1
455	1475	27367	27385	51.4	52.6	3975	27566	27546	50.7	47.6	0.7	200	74.8	44.5	35	52.7
456	1476	10250	10274	51.6	40	3976	10605	10588	51.1	50	0.5	356	74.6	40.4	35	52.6
457	1477	27367	27385	51.4	52.6	3977	27568	27548	50.2	42.9	1.2	202	74.6	44.1	35	52.4
458	1478	27367	27385	51.4	52.6	3978	27571	27551	51.4	42.9	0	205	74.6	43.9	35	52.7
459	1479	8867	8887	52.3	47.6	3979	9376	9355	51	40.9	1.3	510	75.7	41.8	35	53.4
460	1480	27367	27385	51.4	52.6	3980	27576	27555	51	40.9	0.4	210	74.8	44.3	35	52.8
461	1481	27367	27385	51.4	52.6	3981	27579	27558	51.1	40.9	0.3	213	75	44.6	35	52.9
462	1482	18704	18724	50.8	47.6	3982	19215	19194	50.2	40.9	0.5	512	75.5	41.2	35	53
463	1483	18704	18724	50.8	47.6	3983	19217	19196	50.2	40.9	0.5	514	75.5	41.2	35	53
464	1484	18696	18715	51.7	50	3984	19215	19194	50.2	40.9	1.5	520	75.6	41.3	35	53.1
465	1485	27365	27384	52.6	50	3985	27464	27443	54	45.5	1.4	100	70.8	43	35	50.4
466	1486	18696	18715	51.7	50	3986	19217	19196	50.2	40.9	1.5	522	75.6	41.4	35	53.1
467	1487	3361	3381	50.5	42.9	3987	3646	3625	52	40.9	1.5	286	75.5	43.7	35	53.1
468	1488	3361	3381	50.5	42.9	3988	3647	3628	50.6	45	0.1	287	75.6	43.9	35	53.1
469	1489	3782	3801	51.3	50	3989	4445	4425	50.6	42.9	0.7	664	75.5	40.5	35	53.1
470	1490	13039	13058	51.8	50	3990	13155	13137	52.1	52.6	0.3	117	73.4	47	35	52
471	1491	3782	3801	51.3	50	3991	4444	4424	50.6	42.9	0.7	663	75.5	40.6	35	53.1
472	1492	13040	13059	50.9	50	3992	13747	13726	50.8	40.9	0.1	708	76.6	43.1	35	54
473	1493	2223	2243	50.2	42.9	3993	2747	2727	50	42.9	0.2	525	76.9	44.6	35	53.9
474	1494	9929	9946	50	50	3994	10449	10431	50.9	47.4	0.9	521	75.4	40.9	35	52.9
475	1495	18077	18097	51.5	47.6	3995	18702	18685	50.2	50	1.4	626	76.2	42.3	35	53.5

476	1496	3360	3379	50.7	45	3996	3646	3625	52	40.9	1.3	287	75.4	43.6	35	53.1
477	1497	26708	26731	54.2	41.7	3997	27463	27443	52.7	42.9	1.5	756	75.9	41.1	35	54
478	1498	26708	26731	54.2	41.7	3998	27464	27444	53	42.9	1.2	757	75.9	41.2	35	54.1
479	1499	26708	26731	54.2	41.7	3999	27464	27445	52.4	45	1.8	757	75.9	41.2	35	54
480	1500	4	22	52.3	52.6	4000	713	695	50.7	47.4	1.6	710	79	49	35	55.7
481	1501	26708	26727	50	45	4001	27462	27443	51.4	45	1.3	755	75.9	41.2	35	53.2
482	1502	3360	3380	51.4	42.9	4002	3646	3625	52	40.9	0.6	287	75.4	43.6	35	53.3
483	1503	26708	26727	50	45	4003	27463	27445	50.8	42.1	0.8	756	75.9	41.1	35	53.2
484	1504	988	1006	52.2	52.6	4004	1493	1474	50.8	45	1.5	506	76.5	43.7	35	53.9
485	1505	12352	12375	52.9	41.7	4005	12993	12975	51.4	47.4	1.5	642	76.5	43	35	54
486	1506	3360	3380	51.4	42.9	4006	3647	3628	50.6	45	0.8	288	75.5	43.8	35	53.1
487	1507	18074	18094	51.1	42.9	4007	18702	18685	50.2	50	0.9	629	76.2	42.3	35	53.5
488	1508	3360	3380	51.4	42.9	4008	3650	3631	53.1	50	1.7	291	75.6	44	35	53.5
489	1509	8374	8395	52.4	45.5	4009	8928	8911	51.9	50	0.6	555	75.1	40	35	53.2
490	1510	9929	9946	50	50	4010	10449	10428	51.9	40.9	1.9	521	75.4	40.9	35	52.9
491	1511	26421	26441	51.5	42.9	4011	27132	27111	50.3	40.9	1.2	712	77.1	44.2	35	54.2
492	1512	10250	10274	51.6	40	4012	10356	10336	52.4	47.6	0.8	107	70.8	42.1	35	50.2
493	1513	18074	18093	50.3	45	4013	18702	18685	50.2	50	0.2	629	76.2	42.3	35	53.5
494	1514	18017	18036	54.8	55	4014	18220	18202	54.8	52.6	0	204	74.3	43.1	35	53.5
495	1515	18017	18036	54.8	55	4015	18225	18206	53.7	50	1.1	209	74.3	43.1	35	53.2
496	1516	18017	18036	54.8	55	4016	18232	18210	54.4	47.8	0.4	216	74.6	43.5	35	53.6
497	1517	18017	18036	54.8	55	4017	18234	18214	53.4	47.6	1.4	218	74.7	43.6	35	53.4
498	1518	18017	18036	54.8	55	4018	18235	18215	54.2	52.4	0.6	219	74.8	43.8	35	53.7
499	1519	18017	18036	54.8	55	4019	18443	18424	55.9	55	1.1	427	76	43.1	35	54.7
500	1520	18012	18031	53.2	55	4020	18220	18202	54.8	52.6	1.7	209	74.5	43.5	35	53.2
501	1521	18013	18031	50.6	52.6	4021	18223	18206	51.8	50	1.1	211	74.4	43.1	35	52.4
502	1522	18013	18031	50.6	52.6	4022	18231	18210	52.2	45.5	1.6	219	74.6	43.4	35	52.5
503	1523	18013	18031	50.6	52.6	4023	18233	18214	52	50	1.4	221	74.8	43.9	35	52.7
504	1524	18013	18031	50.6	52.6	4024	18233	18215	51.3	52.6	0.7	221	74.8	43.9	35	52.7
505	1525	18013	18031	50.6	52.6	4025	18662	18641	50.4	40.9	0.2	650	76.3	42.6	35	53.7
506	1526	18009	18029	53.3	52.4	4026	18220	18202	54.8	52.6	1.6	212	74.5	43.4	35	53.2
507	1527	18011	18029	51.3	52.6	4027	18223	18206	51.8	50	0.5	213	74.4	43.2	35	52.6
508	1528	18011	18029	51.3	52.6	4028	18231	18210	52.2	45.5	0.9	221	74.7	43.4	35	52.8
509	1529	18011	18029	51.3	52.6	4029	18233	18214	52	50	0.7	223	74.9	43.9	35	52.9
510	1530	18011	18029	51.3	52.6	4030	18233	18215	51.3	52.6	0	223	74.9	43.9	35	52.9
511	1531	16374	16397	52.8	41.7	4031	16774	16751	53.6	41.7	0.8	401	75	40.9	35	53.4
512	1532	16378	16397	50.4	45	4032	16780	16760	51.4	42.9	1	403	75	40.9	35	52.7
513	1533	2223	2243	50.2	42.9	4033	2997	2976	51.4	40.9	1.2	775	76.7	43.1	35	53.9
514	1534	2428	2447	51.5	50	4034	3082	3058	52.3	40	0.8	655	76.3	42.6	35	54
515	1535	16548	16566	54.9	52.6	4035	16774	16751	53.6	41.7	1.3	227	73.9	41.4	35	52.9
516	1536	16367	16386	51.4	50	4036	16774	16752	52.2	43.5	0.8	408	75	40.9	35	53
517	1537	3230	3249	50.1	45	4037	3497	3478	51.3	50	1.2	268	74.4	41.4	35	52.2
518	1538	8221	8240	52.4	50	4038	8920	8901	53.4	50	1	700	75.3	40	35	53.6
519	1539	3232	3252	51.1	47.6	4039	3500	3481	51.2	50	0.1	269	74.5	41.6	35	52.5
520	1540	3232	3252	51.1	47.6	4040	3497	3478	51.3	50	0.2	266	74.5	41.7	35	52.6
521	1541	16367	16386	51.4	50	4041	17111	17090	51.1	40.9	0.3	745	76.3	42.1	35	53.8
522	1542	16366	16385	52.9	55	4042	16774	16751	53.6	41.7	0.8	409	75.1	41.1	35	53.5
523	1543	9930	9948	51.5	52.6	4043	10670	10649	51.3	40.9	0.2	741	75.8	40.9	35	53.5
524	1544	12370	12388	50.1	47.4	4044	12996	12977	50.2	40	0.2	627	76.4	42.7	35	53.6
525	1545	25354	25372	50.9	52.6	4045	25650	25631	51.3	45	0.4	297	74.5	41.1	35	52.5
526	1546	25354	25372	50.9	52.6	4046	25651	25634	50.4	50	0.5	298	74.6	41.3	35	52.4

527	1547	25354	25372	50.9	52.6	4047	25772	25753	51.9	50	1	419	74.8	40.3	35	52.8
528	1548	1402	1422	50.2	42.9	4048	1501	1482	50.5	45	0.3	100	72	46	35	50.6
529	1549	25354	25372	50.9	52.6	4049	25831	25809	51.4	43.5	0.5	478	75	40.2	35	52.8
530	1550	3797	3815	50.9	47.4	4050	4434	4416	51.5	52.6	0.5	638	75.4	40.3	35	53.1
531	1551	25354	25372	50.9	52.6	4051	25831	25810	50.7	45.5	0.2	478	75	40.2	35	52.8
532	1552	3797	3815	50.9	47.4	4052	4435	4417	50.5	52.6	0.5	639	75.4	40.4	35	53
533	1553	24481	24500	50.1	45	4053	24938	24921	50.4	50	0.3	458	75.6	41.9	35	53.1
534	1554	25348	25366	51.2	47.4	4054	25831	25809	51.4	43.5	0.2	484	75	40.3	35	53
535	1555	25348	25366	51.2	47.4	4055	25831	25810	50.7	45.5	0.4	484	75	40.3	35	52.8
536	1556	24419	24440	52.3	45.5	4056	25080	25062	53.5	52.6	1.2	662	75.7	41.1	35	53.8
537	1557	24420	24440	50.8	42.9	4057	24527	24508	50.5	45	0.3	108	70.7	41.7	35	49.8
538	1558	25348	25365	50.4	50	4058	25650	25631	51.3	45	0.9	303	74.6	41.3	35	52.4
539	1559	25348	25365	50.4	50	4059	25651	25634	50.4	50	0.1	304	74.7	41.4	35	52.5
540	1560	25348	25365	50.4	50	4060	25831	25809	51.4	43.5	1	484	75	40.3	35	52.7
541	1561	25348	25365	50.4	50	4061	25831	25810	50.7	45.5	0.3	484	75	40.3	35	52.7
542	1562	28618	28636	52.5	52.6	4062	29298	29280	51.4	52.6	1.1	681	78.3	47.3	35	55.3
543	1563	8867	8887	52.3	47.6	4063	9317	9297	50.5	42.9	1.8	451	75.5	41.7	35	53.1
544	1564	28820	28838	53.7	52.6	4064	29301	29282	55.3	55	1.6	482	77.1	45.4	35	55.2
545	1565	27365	27385	53.2	47.6	4065	27464	27443	54	45.5	0.8	100	70.8	43	35	50.6
546	1566	28820	28839	54.3	50	4066	29306	29288	53.5	52.6	0.8	487	77.1	45.4	35	55.1
547	1567	28820	28839	54.3	50	4067	29301	29282	55.3	55	1	482	77.1	45.4	35	55.4
548	1568	28821	28840	51.8	45	4068	29306	29288	53.5	52.6	1.7	486	77.1	45.3	35	54.6
549	1569	27370	27389	50.1	45	4069	27675	27656	50	40	0.1	306	74.2	40.2	35	52
550	1570	28820	28840	54.8	47.6	4070	29301	29282	55.3	55	0.4	482	77.1	45.4	35	55.5
551	1571	27370	27389	50.1	45	4071	27674	27654	51.9	42.9	1.8	305	74.2	40.3	35	52.1
552	1572	2429	2447	50.2	47.4	4072	3188	3167	50.2	40.9	0	760	76.6	42.9	35	53.8
553	1573	27375	27392	50	50	4073	27675	27656	50	40	0	301	74.1	40.2	35	52
554	1574	27375	27392	50	50	4074	27674	27654	51.9	42.9	1.9	300	74.2	40.3	35	52
555	1575	19795	19814	50.4	45	4075	19916	19895	50.2	40.9	0.2	122	71.8	42.6	35	50.5
556	1576	3168	3189	51	45.5	4076	3646	3625	52	40.9	1.1	479	75.7	42	35	53.4
557	1577	3168	3189	51	45.5	4077	3647	3628	50.6	45	0.4	480	75.8	42.1	35	53.3
558	1578	18011	18029	51.3	52.6	4078	18662	18641	50.4	40.9	0.9	652	76.3	42.6	35	53.7
559	1579	985	1004	51.1	50	4079	1493	1474	50.8	45	0.3	509	76.5	43.6	35	53.9
560	1580	12965	12985	51.2	42.9	4080	13547	13528	50.2	45	0.9	583	77.3	45.3	35	54.3
561	1581	2427	2445	52.1	52.6	4081	3188	3167	50.2	40.9	1.9	762	76.7	43	35	53.8
562	1582	3360	3381	52.1	40.9	4082	3650	3631	53.1	50	1	291	75.6	44	35	53.7
563	1583	12726	12746	51.3	47.6	4083	12911	12892	50.5	50	0.8	186	73.5	41.9	35	51.7
564	1584	19800	19817	50.4	50	4084	19917	19896	50.9	45.5	0.5	118	71.9	43.2	35	50.6
565	1585	1402	1426	54.1	40	4085	1501	1478	54.6	41.7	0.5	100	72	46	35	51.8
566	1586	2427	2445	52.1	52.6	4086	3082	3058	52.3	40	0.2	656	76.4	42.7	35	54.2
567	1587	8867	8887	52.3	47.6	4087	9257	9238	50.5	45	1.8	391	75.1	41.2	35	52.8
568	1588	8867	8887	52.3	47.6	4088	9249	9231	50.8	47.4	1.5	383	75.2	41.5	35	53
569	1589	8374	8394	51	42.9	4089	8928	8911	51.9	50	0.8	555	75.1	40	35	53
570	1590	8867	8887	52.3	47.6	4090	9249	9230	51.5	45	0.8	383	75.2	41.5	35	53.2
571	1591	28964	28984	54.3	52.4	4091	29301	29282	55.3	55	1	338	76.5	45.3	35	54.9
572	1592	8867	8887	52.3	47.6	4092	9249	9229	53	47.6	0.6	383	75.2	41.5	35	53.4
573	1593	12962	12980	50.7	47.4	4093	13547	13528	50.2	45	0.5	586	77.4	45.4	35	54.3
574	1594	9931	9950	50.2	45	4094	10605	10588	51.1	50	0.9	675	75.8	41.2	35	53.2
575	1595	19801	19819	53.2	52.6	4095	19918	19896	52.2	43.5	1	118	71.9	43.2	35	51.1
576	1596	9055	9079	52.8	40	4096	9376	9355	51	40.9	1.8	322	75.1	42.2	35	53
577	1597	19878	19899	50.5	40.9	4097	20033	20016	50.4	50	0.1	156	73.4	43.6	35	51.6

578	1598	17608	17628	50.9	42.9	4098	18233	18214	52	50	1.1	626	75.3	40.3	35	53.1
579	1599	17608	17627	50.2	45	4099	18233	18214	52	50	1.8	626	75.3	40.3	35	52.9
580	1600	29179	29199	51.4	42.9	4100	29358	29339	52.8	50	1.4	180	74.8	45.6	35	52.9
581	1601	29182	29202	51.2	42.9	4101	29358	29339	52.8	50	1.6	177	74.6	45.2	35	52.7
582	1602	4	22	52.3	52.6	4102	253	233	51.8	47.6	0.5	250	76.2	46.4	35	54
583	1603	8221	8240	52.4	50	4103	8920	8902	52.8	52.6	0.3	700	75.3	40	35	53.6
584	1604	16554	16572	53.7	52.6	4104	16774	16751	53.6	41.7	0.1	221	73.7	41.2	35	52.8
585	1605	16555	16572	50.3	50	4105	16711	16691	51	42.9	0.7	157	73.4	43.3	35	51.6
586	1606	29186	29205	50.1	40	4106	29412	29393	50.3	45	0.3	227	75.4	44.9	35	52.9
587	1607	2429	2447	50.2	47.4	4107	3052	3033	50.3	50	0.1	624	76.3	42.6	35	53.6
588	1608	29182	29205	54.6	41.7	4108	29358	29339	52.8	50	1.7	177	74.6	45.2	35	53.2
589	1609	4	22	52.3	52.6	4109	255	235	51.3	47.6	1	252	76.3	46.4	35	53.9
590	1610	3230	3249	50.1	45	4110	3500	3481	51.2	50	1.1	271	74.4	41.3	35	52.2
591	1611	13040	13059	50.9	50	4111	13177	13156	50.4	40.9	0.5	138	73.7	45.7	35	51.8
592	1612	16551	16568	51.1	50	4112	17039	17022	51.4	50	0.3	489	75.8	42.1	35	53.5
593	1613	19995	20012	50.4	50	4113	20615	20597	50.6	47.4	0.2	621	75.3	40.1	35	52.9
594	1614	19995	20013	51.8	52.6	4114	20615	20597	50.6	47.4	1.2	621	75.3	40.1	35	53
595	1615	12370	12388	50.1	47.4	4115	12993	12975	51.4	47.4	1.3	624	76.4	42.9	35	53.6
596	1616	8374	8393	51.2	45	4116	8928	8911	51.9	50	0.7	555	75.1	40	35	53
597	1617	24174	24194	50.9	42.9	4117	24936	24919	51.8	50	0.8	763	75.8	41	35	53.5
598	1618	24179	24198	51	45	4118	24936	24919	51.8	50	0.7	758	75.8	41	35	53.5
599	1619	7679	7698	50.6	50	4119	8049	8032	50.4	50	0.2	371	75.4	42.3	35	53
600	1620	13177	13197	50.3	42.9	4120	13320	13300	51.4	47.6	1.1	144	73.2	43.8	35	51.4
601	1621	24179	24200	53.3	40.9	4121	24934	24913	53.4	45.5	0.2	756	75.8	41	35	54.2
602	1622	9927	9945	50.8	52.6	4122	10670	10649	51.3	40.9	0.5	744	75.7	40.9	35	53.4
603	1623	2427	2445	52.1	52.6	4123	3052	3033	50.3	50	1.8	626	76.4	42.8	35	53.6
604	1624	24418	24436	50	47.4	4124	24527	24507	51	42.9	1	110	71.3	42.7	35	50
605	1625	24417	24436	52.6	50	4125	24517	24494	53.2	41.7	0.6	101	71.1	43.6	35	50.6
606	1626	8375	8396	51.8	45.5	4126	8929	8911	53.4	52.6	1.6	555	75.1	40	35	53.2
607	1627	24418	24439	52.9	45.5	4127	25080	25062	53.5	52.6	0.6	663	75.8	41.2	35	54
608	1628	18074	18094	51.1	42.9	4128	18662	18641	50.4	40.9	0.6	589	76.2	42.6	36	53.6
609	1629	18074	18094	51.1	42.9	4129	18632	18611	50.2	40.9	0.9	559	76.2	42.8	36	53.5
610	1630	13231	13251	50.1	42.9	4130	13545	13527	50.3	52.6	0.2	315	77	47	36	54
611	1631	7400	7417	50.2	50	4131	8188	8169	50.5	45	0.3	789	76.4	42.2	36	53.6
612	1632	3792	3811	54	55	4132	4446	4424	52.4	43.5	1.6	655	75.5	40.6	36	53.7
613	1633	25782	25805	52.1	41.7	4133	26182	26161	51.2	40.9	0.9	401	74.7	40.1	36	52.7
614	1634	13230	13251	52.4	45.5	4134	13545	13526	52.9	55	0.5	316	77.1	47.2	36	54.8
615	1635	985	1004	51.1	50	4135	1480	1462	51.6	47.4	0.5	496	76.4	43.5	36	53.9
616	1636	7400	7417	50.2	50	4136	8049	8032	50.4	50	0.2	650	76.1	42.2	36	53.5
617	1637	13176	13197	52.7	45.5	4137	13545	13526	52.9	55	0.2	370	77	46.2	36	54.8
618	1638	25782	25806	53.5	40	4138	26183	26162	52.8	45.5	0.7	402	74.7	40.3	36	53.3
619	1639	13176	13196	51.4	47.6	4139	13547	13528	50.2	45	1.2	372	76.9	46	36	54
620	1640	12938	12956	50.1	47.4	4140	13155	13138	50.4	50	0.3	218	75.4	45.4	36	52.9
621	1641	18080	18099	53	50	4141	18712	18693	54.8	55	1.9	633	76.3	42.7	36	54.4
622	1642	9140	9159	50.1	45	4142	9375	9354	50.4	40.9	0.3	236	74.6	42.8	36	52.3
623	1643	7725	7742	50	50	4143	8054	8035	50.4	50	0.4	330	75	41.8	36	52.6
624	1644	9922	9941	51.3	50	4144	10455	10435	50.5	42.9	0.8	534	75.3	40.6	36	52.9
625	1645	12938	12957	50.9	45	4145	13155	13138	50.4	50	0.5	218	75.4	45.4	36	53
626	1646	12366	12384	51.7	52.6	4146	12996	12977	50.2	40	1.4	631	76.4	42.8	36	53.6
627	1647	7617	7636	50.9	50	4147	8049	8032	50.4	50	0.6	433	75.7	42.3	36	53.2
628	1648	2671	2692	52.1	40.9	4148	3188	3167	50.2	40.9	2	518	75.6	41.5	36	53.1

629	1649	26039	26057	52.6	52.6	4149	26183	26164	51	45	1.6	145	71.9	40.7	36	50.8
630	1650	11540	11557	50.4	50	4150	11727	11708	50.4	45	0.1	188	73.1	41	36	51.4
631	1651	12962	12980	50.7	47.4	4151	13545	13527	50.3	52.6	0.4	584	77.4	45.5	36	54.4
632	1652	12961	12980	53.2	50	4152	13545	13526	52.9	55	0.4	585	77.5	45.6	36	55.2
633	1653	9055	9079	52.8	40	4153	9369	9350	51.5	50	1.4	315	75.3	42.9	36	53.3
634	1654	12965	12985	51.2	42.9	4154	13545	13527	50.3	52.6	0.9	581	77.4	45.4	36	54.3
635	1655	26039	26058	54	55	4155	26693	26674	54.8	55	0.8	655	75.7	41.1	36	54.3
636	1656	26039	26058	54	55	4156	26692	26673	52.6	50	1.4	654	75.7	41	36	53.8
637	1657	26039	26058	54	55	4157	26688	26669	52.1	45	2	650	75.6	40.8	36	53.6
638	1658	26039	26058	54	55	4158	26684	26666	53.4	52.6	0.6	646	75.6	40.9	36	54.1
639	1659	26039	26058	54	55	4159	26683	26665	52.7	52.6	1.4	645	75.6	40.9	36	53.8
640	1660	12965	12985	51.2	42.9	4160	13545	13526	52.9	55	1.7	581	77.4	45.4	36	54.6
641	1661	26039	26058	54	55	4161	26183	26162	52.8	45.5	1.2	145	71.9	40.7	36	51.3
642	1662	9055	9079	52.8	40	4162	9365	9347	53	52.6	0.2	311	75.3	42.8	36	53.6
643	1663	19795	19814	50.4	45	4163	19922	19902	50	42.9	0.4	128	72.2	43	36	50.7
644	1664	12965	12988	54	41.7	4164	13545	13526	52.9	55	1.2	581	77.4	45.4	36	55.1
645	1665	26040	26061	56.4	54.5	4165	26693	26674	54.8	55	1.6	654	75.7	41.1	36	54.6
646	1666	26040	26061	56.4	54.5	4166	26693	26673	55.3	52.4	1.1	654	75.7	41.1	36	54.7
647	1667	26040	26061	56.4	54.5	4167	26690	26669	56.3	50	0.1	651	75.7	41	36	55
648	1668	26040	26061	56.4	54.5	4168	26685	26666	54.8	55	1.6	646	75.7	41	36	54.5
649	1669	26040	26061	56.4	54.5	4169	26685	26665	55.3	52.4	1.1	646	75.7	41	36	54.7
650	1670	18011	18031	54.5	52.4	4170	18443	18424	55.9	55	1.4	433	76.1	43.2	36	54.7
651	1671	7876	7895	51.5	45	4171	8049	8032	50.4	50	1.2	174	73.2	42	36	51.5
652	1672	3230	3249	50.1	45	4172	3646	3625	52	40.9	1.9	417	75.2	41.2	36	52.8
653	1673	19795	19814	50.4	45	4173	19920	19899	50.2	40.9	0.3	126	72.1	42.9	36	50.6
654	1674	12366	12384	51.7	52.6	4174	12993	12975	51.4	47.4	0.3	628	76.5	43	36	54
655	1675	19793	19814	54	50	4175	20544	20524	52.3	47.6	1.7	752	75.4	40	36	53.6
656	1676	12366	12384	51.7	52.6	4176	12911	12892	50.5	50	1.2	546	76.1	42.5	36	53.5
657	1677	7728	7746	51.7	52.6	4177	8188	8168	50.4	42.9	1.3	461	75.6	41.9	36	53.1
658	1678	26421	26441	51.5	42.9	4178	27084	27063	51.6	40.9	0.2	664	77.3	45	36	54.7
659	1679	9929	9946	50	50	4179	10455	10434	51.1	40.9	1.1	527	75.3	40.6	36	52.8
660	1680	26421	26441	51.5	42.9	4180	27083	27062	50.7	40.9	0.8	663	77.4	45.1	36	54.5
661	1681	12236	12256	51.2	42.9	4181	12999	12980	50.6	40	0.6	764	76.4	42.4	36	53.8
662	1682	26421	26441	51.5	42.9	4182	26694	26677	51.4	50	0	274	74.9	42.7	36	53
663	1683	9929	9946	50	50	4183	10183	10166	50.9	50	0.8	255	75.3	43.9	36	52.8
664	1684	12234	12252	50.6	47.4	4184	13000	12981	51.1	45	0.5	767	76.4	42.5	36	53.8
665	1685	8868	8889	50.4	40.9	4185	9254	9236	50.6	47.4	0.2	387	75	41.1	36	52.7
666	1686	9130	9150	51.3	42.9	4186	9597	9577	50.3	42.9	1	468	75.4	41.2	36	52.9
667	1687	9935	9955	50.4	42.9	4187	10605	10588	51.1	50	0.7	671	75.8	41.1	36	53.2
668	1688	26421	26441	51.5	42.9	4188	26587	26569	52	47.4	0.5	167	72.3	40.1	36	51.2
669	1689	9130	9150	51.3	42.9	4189	9597	9576	51	40.9	0.3	468	75.4	41.2	36	53.2
670	1690	26708	26727	50	45	4190	27466	27449	51	50	0.9	759	76	41.4	36	53.3
671	1691	9130	9150	51.3	42.9	4191	9375	9354	50.4	40.9	0.9	246	74.7	42.7	36	52.5
672	1692	10246	10266	50.4	47.6	4192	10608	10589	51	50	0.5	363	74.5	40.2	36	52.4
673	1693	9924	9944	53.1	52.4	4193	10449	10425	54.6	40	1.5	526	75.4	40.9	36	53.8
674	1694	12366	12384	51.7	52.6	4194	12911	12891	51.2	47.6	0.5	546	76.1	42.5	36	53.7
675	1695	26708	26731	54.2	41.7	4195	27466	27448	52.3	52.6	1.9	759	76	41.4	36	54
676	1696	8867	8888	52.7	45.5	4196	9107	9086	51.6	45.5	1.1	241	74.1	41.5	36	52.5
677	1697	9131	9151	50.4	42.9	4197	9597	9577	50.3	42.9	0.1	467	75.4	41.3	36	53
678	1698	9131	9151	50.4	42.9	4198	9597	9576	51	40.9	0.6	467	75.4	41.3	36	53
679	1699	10242	10265	51.2	41.7	4199	10608	10589	51	50	0.3	367	74.5	40.1	36	52.5

680	1700	27361	27380	52.4	55	4200	27468	27451	51.1	50	1.3	108	72.3	45.4	36	51
681	1701	27361	27380	52.4	55	4201	27467	27450	52.1	50	0.3	107	72.4	45.8	36	51.4
682	1702	27361	27380	52.4	55	4202	27466	27449	51	50	1.4	106	72.5	46.2	36	51.1
683	1703	9926	9944	50.5	52.6	4203	10449	10428	51.9	40.9	1.4	524	75.4	40.8	36	53
684	1704	9926	9944	50.5	52.6	4204	10449	10431	50.9	47.4	0.5	524	75.4	40.8	36	53
685	1705	19802	19820	53	52.6	4205	19922	19901	51.5	45.5	1.4	121	72.3	43.8	36	51.2
686	1706	27361	27380	52.4	55	4206	27462	27443	51.4	45	1	102	71.8	45.1	36	50.8
687	1707	10140	10159	52.4	50	4207	10605	10588	51.1	50	1.3	466	75	40.3	36	52.9
688	1708	16366	16384	50.3	52.6	4208	16777	16758	51.5	50	1.2	412	75.1	41	36	52.8
689	1709	16366	16385	52.9	55	4209	16781	16761	51.3	47.6	1.6	416	75.1	41.1	36	53.1
690	1710	985	1008	56.1	50	4210	1484	1464	54.3	47.6	1.8	500	76.4	43.6	36	54.9
691	1711	16366	16385	52.9	55	4211	16777	16758	51.5	50	1.4	412	75.1	41	36	53.1
692	1712	27366	27384	52.2	52.6	4212	27466	27448	52.3	52.6	0.1	101	71.5	44.6	36	50.8
693	1713	985	1008	56.1	50	4213	1483	1462	54.3	45.5	1.8	499	76.4	43.5	36	54.8
694	1714	2823	2844	50.4	45.5	4214	3052	3033	50.3	50	0.2	230	74.1	41.7	36	52
695	1715	3224	3242	50.5	52.6	4215	3504	3485	50.4	45	0.1	281	74.7	42	36	52.5
696	1716	8867	8886	50.7	50	4216	9310	9291	51.2	45	0.5	444	75.4	41.4	36	53.1
697	1717	8867	8886	50.7	50	4217	9254	9236	50.6	47.4	0.1	388	75.1	41.2	36	52.8
698	1718	9349	9367	51.7	52.6	4218	9989	9968	51	40.9	0.7	641	75.4	40.4	36	53.2
699	1719	8867	8887	52.3	47.6	4219	9369	9350	51.5	50	0.8	503	75.8	42.1	36	53.6
700	1720	8867	8887	52.3	47.6	4220	9341	9322	51.1	50	1.2	475	75.7	41.9	36	53.4
701	1721	9926	9944	50.5	52.6	4221	10608	10589	51	50	0.5	683	75.8	41.1	36	53.3
702	1722	7725	7742	50	50	4222	8190	8172	50.3	47.4	0.3	466	75.6	41.8	36	53
703	1723	9131	9151	50.4	42.9	4223	9375	9354	50.4	40.9	0	245	74.7	42.9	36	52.5
704	1724	3055	3075	51.8	47.6	4224	3494	3473	50.4	40.9	1.4	440	76	43	36	53.4
705	1725	7725	7742	50	50	4225	8189	8170	50.6	50	0.6	465	75.6	41.9	36	53.1
706	1726	2823	2844	50.4	45.5	4226	3056	3038	50.8	52.6	0.3	234	74.2	41.9	36	52.2
707	1727	12370	12388	50.1	47.4	4227	13155	13138	50.4	50	0.3	786	76.8	43.4	36	53.9
708	1728	3055	3075	51.8	47.6	4228	3209	3189	50.5	47.6	1.3	155	74.1	45.2	36	52.1
709	1729	8867	8887	52.3	47.6	4229	9340	9319	50.8	45.5	1.6	474	75.6	41.8	36	53.3
710	1730	27367	27385	51.4	52.6	4230	27466	27448	52.3	52.6	0.9	100	71.6	45	36	50.6
711	1731	14951	14975	52.2	40	4231	15146	15129	50.3	50	1.9	196	73.2	40.8	36	51.4
712	1732	8867	8887	52.3	47.6	4232	9311	9292	50.7	50	1.6	445	75.4	41.6	36	53.1
713	1733	12234	12252	50.6	47.4	4233	12999	12980	50.6	40	0	766	76.4	42.4	36	53.8
714	1734	3055	3076	52.4	45.5	4234	3495	3473	51.8	43.5	0.6	441	76	43.1	36	53.9
715	1735	8867	8887	52.3	47.6	4235	9109	9087	50.5	43.5	1.8	243	74	41.2	36	52.1
716	1736	3055	3076	52.4	45.5	4236	3209	3189	50.5	47.6	2	155	74.1	45.2	36	52.1
717	1737	2671	2692	52.1	40.9	4237	3053	3034	50.3	50	1.8	383	74.7	40.5	36	52.5
718	1738	16981	17000	51.3	50	4238	17501	17481	51.2	42.9	0.1	521	75.9	42.2	36	53.6
719	1739	3796	3814	50.8	52.6	4239	4444	4424	50.6	42.9	0.2	649	75.5	40.5	36	53.1
720	1740	3796	3814	50.8	52.6	4240	4445	4425	50.6	42.9	0.2	650	75.5	40.5	36	53.1
721	1741	27382	27401	50.8	45	4241	27546	27527	51.3	50	0.6	165	73.7	43.6	36	51.9
722	1742	27382	27401	50.8	45	4242	27541	27522	50.1	45	0.6	160	73.4	43.1	36	51.5
723	1743	27383	27403	50.3	42.9	4243	27546	27527	51.3	50	1.1	164	73.5	43.3	36	51.7
724	1744	27383	27403	50.3	42.9	4244	27541	27522	50.1	45	0.1	159	73.2	42.8	36	51.4
725	1745	17789	17811	52.9	43.5	4245	18220	18202	54.8	52.6	1.9	432	74.9	40.5	36	53.4
726	1746	17791	17813	52.9	43.5	4246	18220	18202	54.8	52.6	1.9	430	74.9	40.5	36	53.4
727	1747	18004	18023	51.1	50	4247	18233	18215	51.3	52.6	0.2	230	75	43.9	36	52.9
728	1748	18004	18023	51.1	50	4248	18231	18210	52.2	45.5	1.1	228	74.7	43.4	36	52.8
729	1749	27437	27456	50.2	40	4249	27546	27527	51.3	50	1.1	110	72.4	45.5	36	50.8
730	1750	27437	27456	50.2	40	4250	27541	27522	50.1	45	0.1	105	71.8	44.8	36	50.4

731	1751	18004	18023	51.1	50	4251	18223	18206	51.8	50	0.6	220	74.5	43.2	36	52.6
732	1752	12233	12251	51.1	52.6	4252	12994	12976	50.3	47.4	0.8	762	76.5	42.5	36	53.7
733	1753	7869	7889	52.5	47.6	4253	8192	8172	50.9	42.9	1.6	324	75.2	42.3	36	53
734	1754	3224	3242	50.5	52.6	4254	3503	3484	51.5	50	0.9	280	74.8	42.1	36	52.6
735	1755	3224	3242	50.5	52.6	4255	3500	3481	51.2	50	0.6	277	74.6	41.9	36	52.5
736	1756	3224	3242	50.5	52.6	4256	3497	3478	51.3	50	0.8	274	74.6	42	36	52.5
737	1757	1	22	54.8	50	4257	204	185	56.6	55	1.8	204	75.1	45.1	36	54.1
738	1758	9140	9159	50.1	45	4258	9597	9576	51	40.9	0.9	458	75.3	41.3	36	52.9
739	1759	28179	28200	50.8	40.9	4259	28671	28653	50.2	52.6	0.6	493	79.7	51.7	36	56
740	1760	9140	9159	50.1	45	4260	9560	9540	51.6	42.9	1.5	421	75.2	41.3	36	52.8
741	1761	7728	7746	51.7	52.6	4261	8189	8170	50.6	50	1.1	462	75.7	42	36	53.3
742	1762	9140	9159	50.1	45	4262	9559	9539	50.6	42.9	0.5	420	75.3	41.4	36	52.8
743	1763	12235	12253	50.1	52.6	4263	12998	12979	50.1	45	0	764	76.5	42.5	36	53.7
744	1764	3225	3244	52.4	55	4264	3503	3484	51.5	50	1	279	74.8	42.3	36	52.9
745	1765	14951	14975	52.2	40	4265	15595	15576	50.8	45	1.3	645	75.5	40.6	36	53.2
746	1766	3225	3244	52.4	55	4266	3500	3481	51.2	50	1.3	276	74.7	42	36	52.7
747	1767	12233	12251	51.1	52.6	4267	12999	12980	50.6	40	0.6	767	76.4	42.5	36	53.8
748	1768	3225	3244	52.4	55	4268	3497	3478	51.3	50	1.1	273	74.7	42.1	36	52.8
749	1769	12233	12251	51.1	52.6	4269	13000	12981	51.1	45	0.1	768	76.5	42.6	36	54
750	1770	28395	28414	51.5	45	4270	28671	28653	50.2	52.6	1.3	277	78.5	51.3	36	55.1
751	1771	28395	28414	51.5	45	4271	28671	28652	52.8	55	1.3	277	78.5	51.3	36	55.5
752	1772	9931	9950	50.2	45	4272	10449	10431	50.9	47.4	0.8	519	75.3	40.8	36	52.9
753	1773	12235	12253	50.1	52.6	4273	12994	12976	50.3	47.4	0.2	760	76.4	42.5	36	53.6
754	1774	3359	3379	51.2	42.9	4274	3650	3631	53.1	50	1.9	292	75.6	43.8	36	53.4
755	1775	11543	11562	50.4	40	4275	12258	12238	50.3	42.9	0.2	716	76.1	41.9	36	53.5
756	1776	28396	28416	52.4	47.6	4276	28672	28653	51.8	55	0.5	277	78.6	51.6	36	55.7
757	1777	28396	28416	52.4	47.6	4277	28671	28652	52.8	55	0.4	276	78.6	51.4	36	55.8
758	1778	3229	3248	50.6	50	4278	3647	3628	50.6	45	0	419	75.3	41.5	36	53
759	1779	12235	12253	50.1	52.6	4279	12992	12974	51.2	52.6	1.1	758	76.5	42.6	36	53.7
760	1780	3229	3248	50.6	50	4280	3646	3625	52	40.9	1.4	418	75.3	41.4	36	53
761	1781	3228	3248	52	47.6	4281	3650	3631	53.1	50	1.1	423	75.4	41.6	36	53.5
762	1782	3230	3249	50.1	45	4282	3647	3628	50.6	45	0.5	418	75.3	41.4	36	52.8
763	1783	9931	9950	50.2	45	4283	10449	10428	51.9	40.9	1.8	519	75.3	40.8	36	52.9
764	1784	1402	1422	50.2	42.9	4284	1622	1602	51.6	47.6	1.4	221	76.5	48	36	53.7
765	1785	9922	9941	51.3	50	4285	10608	10589	51	50	0.3	687	75.8	41.2	36	53.5
766	1786	3792	3810	52.9	52.6	4286	4318	4294	54.4	40	1.5	527	75.5	41.2	36	53.8
767	1787	2429	2447	50.2	47.4	4287	3189	3168	51	45.5	0.7	761	76.6	43	36	53.8
768	1788	18008	18029	54.5	50	4288	18443	18424	55.9	55	1.4	436	76	43.1	36	54.7
769	1789	13039	13058	51.8	50	4289	13179	13158	50.4	40.9	1.5	141	74	46.1	36	52
770	1790	942	961	52.8	50	4290	1484	1466	53.1	52.6	0.4	543	76.9	44.4	36	54.7
771	1791	943	961	50.3	47.4	4291	1483	1464	51.3	45	1	541	76.8	44.2	36	53.9
772	1792	28867	28886	53.2	50	4292	29358	29339	52.8	50	0.3	492	76.9	44.9	36	54.8
773	1793	943	961	50.3	47.4	4293	1483	1465	50.5	47.4	0.3	541	76.8	44.2	36	53.9
774	1794	28866	28886	55.4	52.4	4294	29301	29282	55.3	55	0.2	436	77	45.4	36	55.6
775	1795	12352	12375	52.9	41.7	4295	12997	12977	51.8	42.9	1.1	646	76.4	42.9	36	54.1
776	1796	28867	28887	53.7	47.6	4296	29358	29339	52.8	50	0.9	492	76.9	44.9	36	54.8
777	1797	3896	3917	50.7	40.9	4297	4608	4590	51.5	52.6	0.9	713	75.5	40.4	36	53.2
778	1798	6098	6118	50.3	42.9	4298	6486	6467	50.8	45	0.5	389	74.6	40.1	36	52.4
779	1799	28868	28888	51.4	42.9	4299	29358	29339	52.8	50	1.4	491	76.9	44.8	36	54.3
780	1800	8220	8240	54	47.6	4300	8931	8913	55.5	52.6	1.4	712	75.4	40	36	54.1
781	1801	2220	2239	51.3	45	4301	2672	2653	51.6	50	0.4	453	77	45.3	36	54.4

782	1802	12040	12057	50.6	50	4302	12493	12476	50.7	50	0.1	454	76.3	43.6	36	53.7
783	1803	942	960	52.1	52.6	4303	1483	1464	51.3	45	0.8	542	76.8	44.3	36	54.3
784	1804	28868	28889	52	40.9	4304	29358	29339	52.8	50	0.8	491	76.9	44.8	36	54.5
785	1805	942	960	52.1	52.6	4305	1483	1465	50.5	47.4	1.6	542	76.8	44.3	36	54
786	1806	12040	12057	50.6	50	4306	12724	12705	52.4	55	1.8	685	76.6	43.1	36	53.9
787	1807	942	960	52.1	52.6	4307	1484	1466	53.1	52.6	1	543	76.9	44.4	36	54.5
788	1808	11545	11563	50.8	47.4	4308	12253	12235	50.1	52.6	0.7	709	76.2	42.2	36	53.5
789	1809	98	118	50.6	42.9	4309	269	251	51.1	52.6	0.5	172	75	46.5	36	52.8
790	1810	12373	12391	50.8	47.4	4310	12911	12892	50.5	50	0.3	539	76.1	42.5	36	53.5
791	1811	16366	16384	50.3	52.6	4311	16781	16761	51.3	47.6	0.9	416	75.1	41.1	36	52.8
792	1812	9929	9946	50	50	4312	10183	10165	51.7	47.4	1.6	255	75.3	43.9	36	52.8
793	1813	12236	12256	51.2	42.9	4313	13000	12981	51.1	45	0.1	765	76.4	42.5	36	53.9
794	1814	3231	3252	52.7	45.5	4314	3650	3631	53.1	50	0.4	420	75.4	41.7	36	53.7
795	1815	11541	11560	50.1	45	4315	11727	11708	50.4	45	0.3	187	73	40.6	36	51.2
796	1816	3232	3252	51.1	47.6	4316	3494	3473	50.4	40.9	0.7	263	74.3	41.4	36	52.3
797	1817	7725	7743	50.8	47.4	4317	8054	8035	50.4	50	0.4	330	75	41.8	36	52.7
798	1818	28968	28988	50.9	47.6	4318	29358	29339	52.8	50	2	391	76.4	44.5	36	53.9
799	1819	11545	11563	50.8	47.4	4319	12257	12237	51.3	47.6	0.5	713	76.2	42.1	36	53.7
800	1820	24417	24436	52.6	50	4320	25080	25062	53.5	52.6	0.9	664	75.8	41.3	36	53.9
801	1821	28968	28989	51.5	45.5	4321	29358	29339	52.8	50	1.3	391	76.4	44.5	36	54.1
802	1822	3789	3808	53.5	50	4322	4318	4294	54.4	40	0.9	530	75.5	41.1	36	54
803	1823	3232	3252	51.1	47.6	4323	3646	3625	52	40.9	0.9	415	75.3	41.4	36	53.1
804	1824	3232	3252	51.1	47.6	4324	3647	3628	50.6	45	0.5	416	75.3	41.6	36	53
805	1825	28971	28993	51.9	43.5	4325	29306	29288	53.5	52.6	1.6	336	76.2	44.6	36	54
806	1826	24179	24200	53.3	40.9	4326	24818	24797	51.6	40.9	1.6	640	75.8	41.2	36	53.6
807	1827	3231	3251	52	47.6	4327	3650	3631	53.1	50	1.1	420	75.4	41.7	36	53.5
808	1828	9930	9950	52.6	47.6	4328	10449	10425	54.6	40	2	520	75.4	41	36	53.7
809	1829	8866	8885	51.1	45	4329	9254	9236	50.6	47.4	0.5	389	75	41.1	36	52.8
810	1830	2522	2541	51.4	45	4330	2672	2653	51.6	50	0.2	151	75.3	48.3	36	53.2
811	1831	11541	11561	50.9	42.9	4331	12258	12238	50.3	42.9	0.6	718	76.2	41.9	36	53.5
812	1832	3232	3251	50.3	50	4332	3646	3625	52	40.9	1.7	415	75.3	41.4	36	52.9
813	1833	3232	3251	50.3	50	4333	3647	3628	50.6	45	0.3	416	75.3	41.6	36	52.9
814	1834	23843	23863	50.3	42.9	4334	24527	24508	50.5	45	0.2	685	76	41.8	36	53.4
815	1835	21210	21228	53.2	52.6	4335	21317	21293	53.2	40	0	108	71.1	42.6	36	50.8
816	1836	3229	3249	51.4	47.6	4336	3650	3631	53.1	50	1.7	422	75.4	41.7	36	53.3
817	1837	3230	3249	50.1	45	4337	3494	3473	50.4	40.9	0.3	265	74.2	41.1	36	52.1
818	1838	2371	2389	50.3	47.4	4338	2997	2976	51.4	40.9	1.1	627	76.7	43.5	36	53.9
819	1839	29186	29206	51.3	42.9	4339	29298	29280	51.4	52.6	0.1	113	72.8	46	36	51.5
820	1840	9929	9946	50	50	4340	10455	10435	50.5	42.9	0.4	527	75.3	40.6	36	52.8
821	1841	9351	9370	51.2	50	4341	9989	9968	51	40.9	0.2	639	75.4	40.4	36	53.2
822	1842	25348	25365	50.4	50	4342	25772	25753	51.9	50	1.5	425	74.9	40.5	36	52.6
823	1843	1402	1422	50.2	42.9	4343	2103	2082	52	45.5	1.8	702	76.7	43.3	36	53.8
824	1844	9929	9946	50	50	4344	10608	10589	51	50	0.9	680	75.8	41.2	36	53.2
825	1845	9934	9953	50.7	50	4345	10608	10589	51	50	0.3	675	75.8	41.2	36	53.4
826	1846	13176	13196	51.4	47.6	4346	13544	13525	52.6	55	1.2	369	77.1	46.3	36	54.5
827	1847	7725	7743	50.8	47.4	4347	8189	8170	50.6	50	0.2	465	75.6	41.9	36	53.2
828	1848	7725	7743	50.8	47.4	4348	8190	8172	50.3	47.4	0.6	466	75.6	41.8	36	53.1
829	1849	18074	18093	50.3	45	4349	18662	18641	50.4	40.9	0.1	589	76.2	42.6	36	53.6
830	1850	18074	18093	50.3	45	4350	18632	18611	50.2	40.9	0.1	559	76.2	42.8	36	53.5
831	1851	29200	29222	53.2	43.5	4351	29306	29288	53.5	52.6	0.3	107	73.1	47.7	36	52.3
832	1852	25348	25366	51.2	47.4	4352	25545	25526	51.7	45	0.5	198	74.1	42.9	36	52.3

833	1853	25348	25366	51.2	47.4	4353	25545	25525	52.3	42.9	1.1	198	74.1	42.9	36	52.3
834	1854	29200	29223	53.7	41.7	4354	29306	29288	53.5	52.6	0.2	107	73.1	47.7	36	52.3
835	1855	25347	25366	52.7	50	4355	25545	25521	54.5	40	1.8	199	74.2	43.2	36	52.9
836	1856	3792	3811	54	55	4356	4447	4425	53	43.5	1	656	75.5	40.5	36	53.9
837	1857	29200	29224	54.2	40	4357	29306	29288	53.5	52.6	0.7	107	73.1	47.7	36	52.3
838	1858	985	1004	51.1	50	4358	1483	1465	50.5	47.4	0.6	499	76.4	43.5	36	53.7
839	1859	2427	2445	52.1	52.6	4359	3189	3168	51	45.5	1.1	763	76.7	43.1	36	54.1
840	1860	13701	13725	53.6	40	4360	14084	14060	53.6	40	0.1	384	74.6	40.1	36	53.4
841	1861	985	1004	51.1	50	4361	1483	1464	51.3	45	0.2	499	76.4	43.5	36	53.9
842	1862	8794	8813	51.6	45	4362	9559	9539	50.6	42.9	1	766	75.9	41.3	37	53.4
843	1863	3789	3806	50	50	4363	4435	4417	50.5	52.6	0.4	647	75.5	40.5	37	52.9
844	1864	13177	13197	50.3	42.9	4364	13314	13297	51	50	0.6	138	72.8	43.5	37	51.2
845	1865	3791	3808	50	50	4365	4435	4417	50.5	52.6	0.4	645	75.4	40.5	37	52.9
846	1866	9139	9159	52.5	47.6	4366	9364	9346	53.9	52.6	1.4	226	74.9	43.8	37	53.3
847	1867	3226	3245	51.7	55	4367	3494	3473	50.4	40.9	1.3	269	74.5	41.6	37	52.4
848	1868	13040	13059	50.9	50	4368	13314	13297	51	50	0.1	275	75.6	44.4	37	53.3
849	1869	2522	2541	51.4	45	4369	2891	2873	50.8	47.4	0.6	370	76	43.8	37	53.6
850	1870	8865	8884	50.4	45	4370	9245	9226	50	45	0.4	381	74.9	40.9	37	52.6
851	1871	3787	3804	50	50	4371	4434	4416	51.5	52.6	1.4	648	75.4	40.3	37	52.9
852	1872	3226	3245	51.7	55	4372	3646	3625	52	40.9	0.3	421	75.3	41.6	37	53.4
853	1873	3226	3245	51.7	55	4373	3647	3628	50.6	45	1.1	422	75.4	41.7	37	53.1
854	1874	3226	3245	51.7	55	4374	3650	3631	53.1	50	1.4	425	75.5	41.9	37	53.5
855	1875	2387	2405	51.6	52.6	4375	2747	2727	50	42.9	1.6	361	76.9	46	37	53.9
856	1876	18074	18093	50.3	45	4376	18229	18209	50.1	42.9	0.2	156	73.2	42.9	37	51.4
857	1877	13701	13725	53.6	40	4377	14059	14040	52.8	50	0.8	359	74.5	40.1	37	53.1
858	1878	3787	3804	50	50	4378	4435	4417	50.5	52.6	0.4	649	75.4	40.4	37	52.9
859	1879	13040	13059	50.9	50	4379	13323	13304	51.1	45	0.2	284	75.7	44.4	37	53.4
860	1880	3789	3806	50	50	4380	4434	4416	51.5	52.6	1.4	646	75.4	40.4	37	52.9
861	1881	15506	15527	50.8	40.9	4381	16214	16196	51.8	52.6	1	709	75.5	40.3	37	53.2
862	1882	12234	12252	50.6	47.4	4382	12412	12392	50	42.9	0.6	179	73.1	41.3	37	51.3
863	1883	12234	12252	50.6	47.4	4383	12739	12718	51	40.9	0.4	506	75.8	42.1	37	53.4
864	1884	18074	18094	51.1	42.9	4384	18229	18209	50.1	42.9	0.9	156	73.2	42.9	37	51.4
865	1885	18075	18095	50.6	47.6	4385	18223	18206	51.8	50	1.2	149	73.3	43.6	37	51.6
866	1886	13040	13059	50.9	50	4386	13326	13306	50.7	42.9	0.2	287	75.7	44.3	37	53.3
867	1887	18080	18098	51.2	52.6	4387	18233	18215	51.3	52.6	0.1	154	73.9	44.8	37	52.2
868	1888	18080	18098	51.2	52.6	4388	18233	18214	52	50	0.9	154	73.9	44.8	37	52.2
869	1889	18080	18098	51.2	52.6	4389	18231	18210	52.2	45.5	1	152	73.5	44.1	37	51.9
870	1890	18080	18098	51.2	52.6	4390	18223	18206	51.8	50	0.6	144	73.2	43.8	37	51.7
871	1891	18077	18098	52.9	50	4391	18220	18202	54.8	52.6	1.9	144	73.2	43.8	37	52.2
872	1892	18076	18098	54.4	47.8	4392	18443	18424	55.9	55	1.5	368	75.8	43.2	37	54.5
873	1893	3792	3810	52.9	52.6	4393	4436	4417	52.2	50	0.6	645	75.4	40.5	37	53.6
874	1894	3055	3074	51.1	50	4394	3647	3628	50.6	45	0.5	593	76.3	42.7	37	53.7
875	1895	3055	3074	51.1	50	4395	3646	3625	52	40.9	0.9	592	76.2	42.6	37	53.8
876	1896	15506	15527	50.8	40.9	4396	15645	15625	51.1	42.9	0.4	140	71.8	40.7	37	50.6
877	1897	18081	18099	51.2	52.6	4397	18229	18209	50.1	42.9	1.1	149	73.3	43.6	37	51.4
878	1898	13039	13058	51.8	50	4398	13155	13138	50.4	50	1.4	117	73.4	47	37	51.6
879	1899	3055	3075	51.8	47.6	4399	3650	3631	53.1	50	1.3	596	76.3	42.8	37	54.1
880	1900	18080	18099	53	50	4400	18223	18205	53.3	52.6	0.4	144	73.2	43.8	37	52.2
881	1901	27361	27380	52.4	55	4401	27579	27558	51.1	40.9	1.3	219	75.4	45.2	37	53.2
882	1902	3221	3239	51.5	52.6	4402	3503	3484	51.5	50	0	283	74.8	42	37	52.9
883	1903	3221	3239	51.5	52.6	4403	3504	3485	50.4	45	1.1	284	74.7	41.9	37	52.5

884	1904	18077	18099	54.4	47.8	4404	18220	18201	56.1	55	1.7	144	73.2	43.8	37	52.6
885	1905	3055	3075	51.8	47.6	4405	3647	3628	50.6	45	1.2	593	76.3	42.7	37	53.7
886	1906	18581	18599	51.4	47.4	4406	18697	18679	51.9	52.6	0.4	117	71	41	37	50.2
887	1907	18616	18636	51.4	47.6	4407	19216	19195	50.2	40.9	1.1	601	75.6	41.1	37	53.1
888	1908	3219	3238	50.7	50	4408	3503	3484	51.5	50	0.8	285	74.8	42.1	37	52.7
889	1909	18696	18715	51.7	50	4409	19216	19195	50.2	40.9	1.5	521	75.5	41.3	37	53
890	1910	3219	3238	50.7	50	4410	3504	3485	50.4	45	0.3	286	74.7	42	37	52.5
891	1911	27366	27384	52.2	52.6	4411	27573	27552	52.3	40.9	0.1	208	74.6	43.8	37	53
892	1912	27366	27384	52.2	52.6	4412	27567	27547	51.1	42.9	1	202	74.6	44.1	37	52.7
893	1913	3055	3075	51.8	47.6	4413	3646	3625	52	40.9	0.2	592	76.2	42.6	37	54
894	1914	18704	18724	50.8	47.6	4414	19216	19195	50.2	40.9	0.5	513	75.5	41.1	37	53
895	1915	16874	16893	52.1	50	4415	17056	17035	51.8	45.5	0.4	183	74.4	44.3	37	52.7
896	1916	12234	12252	50.6	47.4	4416	12739	12719	50.3	42.9	0.3	506	75.8	42.1	37	53.3
897	1917	7728	7746	51.7	52.6	4417	8054	8035	50.4	50	1.2	327	75	41.9	37	52.7
898	1918	15506	15527	50.8	40.9	4418	15647	15628	51	45	0.3	142	71.9	40.8	37	50.7
899	1919	985	1004	51.1	50	4419	1773	1755	51.7	52.6	0.6	789	76.7	43.1	37	54.1
900	1920	3217	3236	51.1	50	4420	3503	3484	51.5	50	0.4	287	74.8	42.2	37	52.8
901	1921	3791	3808	50	50	4421	4434	4416	51.5	52.6	1.4	644	75.4	40.4	37	52.9
902	1922	19794	19813	50	50	4422	19923	19904	50.1	50	0.1	130	72.7	43.8	37	51
903	1923	13039	13058	51.8	50	4423	13178	13157	50.4	40.9	1.5	140	73.8	45.7	37	51.9
904	1924	13033	13051	52.1	52.6	4424	13155	13138	50.4	50	1.7	123	73.7	47.2	37	51.8
905	1925	12233	12251	51.1	52.6	4425	12739	12719	50.3	42.9	0.9	507	75.9	42.2	37	53.3
906	1926	19795	19814	50.4	45	4426	19923	19903	50.9	47.6	0.4	129	72.5	43.4	37	51
907	1927	13177	13197	50.3	42.9	4427	13946	13929	51.5	50	1.2	770	75.9	41	37	53.3
908	1928	3799	3820	52.9	45.5	4428	4318	4294	54.4	40	1.5	520	75.4	41	37	53.7
909	1929	8867	8887	52.3	47.6	4429	9364	9346	53.9	52.6	1.6	498	75.8	42.2	37	53.9
910	1930	1472	1491	51.2	45	4430	2152	2133	50.7	45	0.5	681	76.5	42.9	37	53.8
911	1931	12233	12251	51.1	52.6	4431	12739	12718	51	40.9	0.2	507	75.9	42.2	37	53.5
912	1932	3055	3076	52.4	45.5	4432	3650	3631	53.1	50	0.7	596	76.3	42.8	37	54.2
913	1933	12726	12746	51.3	47.6	4433	13325	13305	50.5	47.6	0.7	600	76.7	43.7	37	53.9
914	1934	8867	8887	52.3	47.6	4434	9316	9296	50.8	42.9	1.5	450	75.4	41.6	37	53.1
915	1935	8867	8887	52.3	47.6	4435	9314	9295	51.1	50	1.2	448	75.5	41.7	37	53.3
916	1936	8867	8887	52.3	47.6	4436	9313	9294	50.4	50	1.9	447	75.5	41.6	37	53
917	1937	3055	3076	52.4	45.5	4437	3647	3628	50.6	45	1.8	593	76.3	42.7	37	53.7
918	1938	13176	13196	51.4	47.6	4438	13312	13294	51	52.6	0.4	137	72.9	43.8	37	51.5
919	1939	12726	12746	51.3	47.6	4439	13155	13138	50.4	50	0.9	430	76.4	44	37	53.7
920	1940	13701	13724	53.1	41.7	4440	14058	14040	51.4	52.6	1.7	358	74.5	40.2	37	52.7
921	1941	8372	8390	50.7	47.4	4441	9101	9081	50.5	47.6	0.2	730	75.5	40.3	37	53.1
922	1942	3055	3076	52.4	45.5	4442	3646	3625	52	40.9	0.4	592	76.2	42.6	37	54.1
923	1943	887	905	50.1	47.4	4443	1493	1474	50.8	45	0.7	607	77.1	44.6	37	54.1
924	1944	1046	1063	50.3	50	4444	1697	1677	51	42.9	0.7	652	76.9	43.9	37	54
925	1945	27378	27397	50.5	45	4445	27675	27656	50	40	0.5	298	74.1	40.3	37	52
926	1946	27378	27397	50.5	45	4446	27674	27654	51.9	42.9	1.4	297	74.2	40.4	37	52.2
927	1947	2671	2692	52.1	40.9	4447	3056	3037	52.1	55	0	386	74.8	40.7	37	53.1
928	1948	1046	1063	50.3	50	4448	1697	1678	50.3	45	0.1	652	76.9	43.9	37	54
929	1949	2387	2405	51.6	52.6	4449	2672	2654	50.9	52.6	0.8	286	77	47.6	37	54.3
930	1950	3792	3810	52.9	52.6	4450	4565	4542	53.9	41.7	1	774	75.6	40.3	37	53.8
931	1951	15506	15527	50.8	40.9	4451	15647	15629	50.3	47.4	0.5	142	71.9	40.8	37	50.5
932	1952	8794	8813	51.6	45	4452	9560	9540	51.6	42.9	0	767	75.9	41.2	37	53.7
933	1953	19801	19819	53.2	52.6	4453	19909	19885	52.5	40	0.7	109	71.4	43.1	37	50.8
934	1954	19988	20006	50.4	47.4	4454	20615	20597	50.6	47.4	0.2	628	75.3	40.1	37	52.9

935	1955	19991	20009	52.8	52.6	4455	20616	20597	52.3	45	0.5	626	75.3	40.1	37	53.5
936	1956	16875	16895	51.6	47.6	4456	17060	17041	51.1	50	0.5	186	74.6	44.6	37	52.6
937	1957	16875	16895	51.6	47.6	4457	17059	17039	50.6	47.6	1	185	74.4	44.3	37	52.4
938	1958	16875	16895	51.6	47.6	4458	17056	17035	51.8	45.5	0.2	182	74.2	44	37	52.5
939	1959	27442	27461	51.5	40	4459	27541	27521	51.7	47.6	0.1	100	71.2	44	37	50.4
940	1960	16875	16896	52.2	45.5	4460	17060	17041	51.1	50	1.1	186	74.6	44.6	37	52.6
941	1961	23841	23859	50.5	52.6	4461	24527	24507	51	42.9	0.5	687	76.1	41.9	37	53.5
942	1962	23841	23859	50.5	52.6	4462	24093	24075	50.9	52.6	0.4	253	76	45.8	37	53.5
943	1963	16875	16896	52.2	45.5	4463	17059	17039	50.6	47.6	1.6	185	74.4	44.3	37	52.4
944	1964	16875	16896	52.2	45.5	4464	17056	17035	51.8	45.5	0.5	182	74.2	44	37	52.6
945	1965	23843	23863	50.3	42.9	4465	24093	24075	50.9	52.6	0.5	251	75.8	45.4	37	53.3
946	1966	16875	16896	52.2	45.5	4466	17041	17023	53.5	52.6	1.3	167	73.8	43.7	37	52.4
947	1967	28187	28205	53.1	52.6	4467	28673	28654	53.5	55	0.5	487	80	52.4	37	57
948	1968	28190	28208	51.7	52.6	4468	28672	28654	50.6	52.6	1.2	483	79.9	52.2	37	56.2
949	1969	24030	24047	50.7	50	4469	24527	24508	50.5	45	0.2	498	75.4	41.2	37	53
950	1970	24031	24050	56.5	55	4470	24816	24792	54.7	40	1.8	786	76.3	42	37	54.9
951	1971	7880	7900	50.3	42.9	4471	8049	8032	50.4	50	0	170	72.8	41.2	37	51.2
952	1972	24096	24119	54.4	41.7	4472	24815	24791	54.5	40	0.1	720	75.8	41	37	54.5
953	1973	17790	17811	51.6	40.9	4473	18233	18214	52	50	0.4	444	75.1	40.8	37	53.2
954	1974	24174	24194	50.9	42.9	4474	24938	24921	50.4	50	0.5	765	75.8	40.9	37	53.3
955	1975	16875	16896	52.2	45.5	4475	17039	17022	51.4	50	0.8	165	73.7	43.6	37	52.1
956	1976	24174	24195	52.5	40.9	4476	24936	24919	51.8	50	0.7	763	75.8	41	37	53.7
957	1977	24179	24198	51	45	4477	24938	24921	50.4	50	0.6	760	75.8	40.9	37	53.3
958	1978	16875	16896	52.2	45.5	4478	17038	17021	50.7	50	1.6	164	73.8	43.9	37	52
959	1979	24180	24199	50.3	40	4479	24936	24919	51.8	50	1.5	757	75.8	41	37	53.2
960	1980	2823	2844	50.4	45.5	4480	3186	3165	50.4	40.9	0	364	75.5	42.6	37	53.1
961	1981	10142	10163	51.3	40.9	4481	10608	10589	51	50	0.3	467	74.9	40	37	52.8
962	1982	1046	1063	50.3	50	4482	1483	1464	51.3	45	0.9	438	76.2	43.6	37	53.6
963	1983	17388	17408	50.7	42.9	4483	17501	17481	51.2	42.9	0.6	114	70.5	40.4	37	49.7
964	1984	24179	24200	53.3	40.9	4484	24740	24717	52.5	41.7	0.8	562	76	42.2	37	54
965	1985	24379	24398	55	55	4485	25088	25070	54.5	52.6	0.5	710	75.9	41.3	37	54.6
966	1986	24379	24398	55	55	4486	25087	25069	53.7	52.6	1.3	709	75.9	41.3	37	54.3
967	1987	16874	16893	52.1	50	4487	17059	17039	50.6	47.6	1.5	186	74.6	44.6	37	52.5
968	1988	24380	24399	55	55	4488	25088	25070	54.5	52.6	0.5	709	75.9	41.3	37	54.6
969	1989	28522	28542	50.2	42.9	4489	28671	28653	50.2	52.6	0	150	76.2	50.7	37	53.5
970	1990	16874	16893	52.1	50	4490	17060	17041	51.1	50	1	187	74.7	44.9	37	52.7
971	1991	24380	24399	55	55	4491	25087	25069	53.7	52.6	1.3	708	75.9	41.4	37	54.4
972	1992	17608	17627	50.2	45	4492	18239	18220	50	45	0.2	632	75.3	40.2	37	52.8
973	1993	17608	17627	50.2	45	4493	18238	18219	50.3	45	0.1	631	75.3	40.3	37	52.9
974	1994	1046	1063	50.3	50	4494	1483	1465	50.5	47.4	0.2	438	76.2	43.6	37	53.6
975	1995	17608	17628	50.9	42.9	4495	18239	18220	50	45	0.9	632	75.3	40.2	37	52.8
976	1996	17608	17628	50.9	42.9	4496	18238	18219	50.3	45	0.7	631	75.3	40.3	37	52.9
977	1997	8063	8084	51.4	45.5	4497	8188	8169	50.5	45	0.9	126	72.1	42.9	37	50.7
978	1998	12236	12256	51.2	42.9	4498	12739	12718	51	40.9	0.2	504	75.8	42.1	37	53.5
979	1999	13176	13196	51.4	47.6	4499	13325	13305	50.5	47.6	0.8	150	73.4	44	37	51.7
980	2000	2371	2389	50.3	47.4	4500	2749	2728	50.3	45.5	0	379	76.9	45.9	37	54.1
981	2001	9402	9420	51.3	47.4	4501	9989	9968	51	40.9	0.4	588	75.4	40.5	37	53.1
982	2002	9931	9950	50.2	45	4502	10183	10166	50.9	50	0.7	253	75.2	43.9	37	52.8
983	2003	2387	2405	51.6	52.6	4503	2997	2976	51.4	40.9	0.2	611	76.6	43.5	37	54.2
984	2004	3788	3805	50	50	4504	4435	4417	50.5	52.6	0.4	648	75.4	40.4	37	52.9
985	2005	26039	26057	52.6	52.6	4505	26650	26630	51.4	42.9	1.2	612	75.3	40.4	37	53.3

986	2006	2371	2389	50.3	47.4	4506	3053	3034	50.3	50	0	683	76.7	43.3	37	53.9
987	2007	3	21	53.4	52.6	4507	315	296	51.9	50	1.5	313	76.9	46.6	37	54.5
988	2008	2371	2389	50.3	47.4	4508	3056	3037	52.1	55	1.7	686	76.7	43.4	37	53.9
989	2009	13040	13059	50.9	50	4509	13155	13137	52.1	52.6	1.2	116	73.2	46.6	37	51.6
990	2010	9931	9950	50.2	45	4510	10183	10165	51.7	47.4	1.5	253	75.2	43.9	37	52.8
991	2011	3788	3805	50	50	4511	4434	4416	51.5	52.6	1.4	647	75.4	40.3	37	52.9
992	2012	13176	13196	51.4	47.6	4512	13946	13929	51.5	50	0.2	771	75.9	41.1	37	53.6
993	2013	3772	3792	51.2	42.9	4513	4444	4424	50.6	42.9	0.7	673	75.6	40.7	37	53.2
994	2014	13176	13196	51.4	47.6	4514	13320	13300	51.4	47.6	0	145	73.3	44.1	37	51.9
995	2015	8861	8880	50.2	45	4515	9245	9226	50	45	0.1	385	74.9	40.8	37	52.5
996	2016	8868	8889	50.4	40.9	4516	9310	9291	51.2	45	0.8	443	75.3	41.3	37	52.9
997	2017	16366	16384	50.3	52.6	4517	16774	16752	52.2	43.5	1.9	409	75.1	41.1	37	52.8
998	2018	9934	9953	50.7	50	4518	10183	10166	50.9	50	0.2	250	75.2	44	37	53
999	2019	9055	9079	52.8	40	4519	9342	9323	52.1	50	0.8	288	75.1	42.7	37	53.3
1000	2020	8868	8889	50.4	40.9	4520	9249	9231	50.8	47.4	0.4	382	75.1	41.4	37	52.8
1001	2021	16366	16385	52.9	55	4521	16774	16752	52.2	43.5	0.6	409	75.1	41.1	37	53.3
1002	2022	8868	8889	50.4	40.9	4522	9249	9230	51.5	45	1.1	382	75.1	41.4	37	52.8
1003	2023	9934	9953	50.7	50	4523	10183	10165	51.7	47.4	0.9	250	75.2	44	37	53
1004	2024	25772	25793	52.4	40.9	4524	26183	26163	51.7	42.9	0.7	412	74.8	40.3	37	53
1005	2025	13039	13057	51.1	52.6	4525	13155	13138	50.4	50	0.7	117	73.4	47	37	51.6
1006	2026	25771	25790	51.1	45	4526	26183	26164	51	45	0.1	413	74.8	40.4	37	52.8
1007	2027	25769	25786	50.3	50	4527	26183	26163	51.7	42.9	1.4	415	74.9	40.5	37	52.6
1008	2028	3794	3812	52.9	52.6	4528	4436	4417	52.2	50	0.6	643	75.4	40.4	37	53.6
1009	2029	887	905	50.1	47.4	4529	1480	1462	51.6	47.4	1.5	594	77.1	44.6	37	54.1
1010	2030	3794	3812	52.9	52.6	4530	4434	4416	51.5	52.6	1.4	641	75.4	40.4	37	53.3
1011	2031	12370	12388	50.1	47.4	4531	12994	12976	50.3	47.4	0.3	625	76.4	42.9	37	53.6
1012	2032	3797	3815	50.9	47.4	4532	4186	4168	51.8	52.6	0.9	390	75.3	41.8	37	53.1
1013	2033	3795	3813	52.1	52.6	4533	4435	4417	50.5	52.6	1.6	641	75.5	40.6	37	53.1
1014	2034	3795	3813	52.1	52.6	4534	4434	4416	51.5	52.6	0.6	640	75.4	40.5	37	53.3
1015	2035	13177	13197	50.3	42.9	4535	13323	13304	51.1	45	0.8	147	73.2	43.5	37	51.4
1016	2036	1046	1064	51.2	47.4	4536	1401	1382	50.6	45	0.6	356	75.6	43	37	53.2
1017	2037	16549	16567	54.9	52.6	4537	17057	17035	53	43.5	1.9	509	75.9	42.2	37	54.1
1018	2038	1046	1064	51.2	47.4	4538	1483	1464	51.3	45	0.1	438	76.2	43.6	37	53.8
1019	2039	16551	16568	51.1	50	4539	17056	17035	51.8	45.5	0.7	506	75.9	42.3	37	53.6
1020	2040	1046	1064	51.2	47.4	4540	1483	1465	50.5	47.4	0.6	438	76.2	43.6	37	53.6
1021	2041	1046	1064	51.2	47.4	4541	1484	1466	53.1	52.6	2	439	76.3	43.7	37	53.9
1022	2042	1046	1064	51.2	47.4	4542	1697	1676	51.7	40.9	0.5	652	76.9	43.9	37	54.2
1023	2043	1046	1064	51.2	47.4	4543	1697	1677	51	42.9	0.2	652	76.9	43.9	37	54.2
1024	2044	16555	16572	50.3	50	4544	17111	17090	51.1	40.9	0.8	557	76.1	42.5	37	53.5
1025	2045	1046	1064	51.2	47.4	4545	1697	1678	50.3	45	0.9	652	76.9	43.9	37	54
1026	2046	1046	1063	50.3	50	4546	1401	1382	50.6	45	0.2	356	75.6	43	37	53.1
1027	2047	3796	3814	50.8	52.6	4547	4435	4417	50.5	52.6	0.3	640	75.4	40.5	37	53.1
1028	2048	12232	12250	51.9	52.6	4548	12993	12975	51.4	47.4	0.5	762	76.5	42.5	37	54
1029	2049	12236	12256	51.2	42.9	4549	12739	12719	50.3	42.9	0.9	504	75.8	42.1	37	53.2
1030	2050	28937	28956	52.4	50	4550	29306	29288	53.5	52.6	1.1	370	76.6	45.1	37	54.4
1031	2051	12232	12250	51.9	52.6	4551	12996	12977	50.2	40	1.7	765	76.4	42.4	37	53.6
1032	2052	3234	3254	51.1	47.6	4552	3504	3485	50.4	45	0.7	271	74.4	41.3	37	52.3
1033	2053	3234	3254	51.1	47.6	4553	3503	3484	51.5	50	0.4	270	74.4	41.5	37	52.5
1034	2054	9922	9941	51.3	50	4554	10670	10649	51.3	40.9	0.1	749	75.8	40.9	37	53.5
1035	2055	3792	3810	52.9	52.6	4555	4434	4416	51.5	52.6	1.4	643	75.4	40.4	37	53.3
1036	2056	3234	3254	51.1	47.6	4556	3494	3473	50.4	40.9	0.6	261	74.1	41	37	52.1

1037	2057	4255	4276	51.7	45.5	4557	4608	4590	51.5	52.6	0.2	354	74.7	40.7	37	52.8
1038	2058	24562	24580	50.1	52.6	4558	24936	24919	51.8	50	1.7	375	75.6	42.7	37	53
1039	2059	24562	24580	50.1	52.6	4559	24938	24921	50.4	50	0.3	377	75.5	42.4	37	53
1040	2060	24562	24580	50.1	52.6	4560	25182	25164	51.4	47.4	1.3	621	75.9	41.7	37	53.3
1041	2061	24559	24579	52	52.4	4561	24936	24919	51.8	50	0.2	378	75.7	42.9	37	53.6
1042	2062	24559	24579	52	52.4	4562	24938	24921	50.4	50	1.6	380	75.6	42.6	37	53.1
1043	2063	1046	1063	50.3	50	4563	1697	1676	51.7	40.9	1.3	652	76.9	43.9	37	54
1044	2064	24482	24503	51.6	40.9	4564	24815	24792	53.4	41.7	1.8	334	75.4	42.8	37	53.4
1045	2065	13177	13197	50.3	42.9	4565	13326	13306	50.7	42.9	0.4	150	73.2	43.3	37	51.4
1046	2066	24480	24502	54.2	47.8	4566	24815	24791	54.5	40	0.3	336	75.6	43.2	37	54.3
1047	2067	17840	17859	50.8	45	4567	18223	18206	51.8	50	1	384	74.7	40.4	37	52.6
1048	2068	24480	24500	53.2	47.6	4568	24815	24792	53.4	41.7	0.2	336	75.6	43.2	37	54
1049	2069	17840	17859	50.8	45	4569	18231	18210	52.2	45.5	1.4	392	74.8	40.6	37	52.7
1050	2070	28821	28840	51.8	45	4570	29298	29279	52.6	55	0.8	478	77	45.2	37	54.6
1051	2071	24418	24440	55	47.8	4571	24517	24494	53.2	41.7	1.8	100	70.8	43	37	50.6
1052	2072	13701	13722	50.4	40.9	4572	14058	14040	51.4	52.6	1	358	74.5	40.2	37	52.4
1053	2073	28821	28840	51.8	45	4573	29358	29339	52.8	50	1	538	77.1	45	37	54.6
1054	2074	17792	17813	51.6	40.9	4574	18233	18214	52	50	0.4	442	75.1	40.7	37	53.1
1055	2075	24420	24440	50.8	42.9	4575	25079	25061	52.7	52.6	1.9	660	75.7	41.1	37	53.3
1056	2076	28821	28839	51.1	47.4	4576	29298	29279	52.6	55	1.5	478	77	45.2	37	54.3
1057	2077	3796	3814	50.8	52.6	4577	4434	4416	51.5	52.6	0.7	639	75.4	40.4	37	53.1
1058	2078	28821	28839	51.1	47.4	4578	29358	29339	52.8	50	1.7	538	77.1	45	37	54.4
1059	2079	28820	28838	53.7	52.6	4579	29298	29279	52.6	55	1.1	479	77.1	45.3	37	54.8
1060	2080	24418	24439	52.9	45.5	4580	25079	25061	52.7	52.6	0.2	662	75.8	41.2	37	54
1061	2081	28820	28838	53.7	52.6	4581	29358	29339	52.8	50	0.9	539	77.1	45.1	37	54.9
1062	2082	27369	27389	52.5	47.6	4582	27468	27451	51.1	50	1.4	100	71.2	44	38	50.3
1063	2083	7725	7742	50	50	4583	8187	8167	50.4	42.9	0.3	463	75.6	41.9	38	53
1064	2084	16549	16567	54.9	52.6	4584	17040	17021	53.4	50	1.5	492	75.9	42.3	38	54.2
1065	2085	3221	3239	51.5	52.6	4585	3500	3481	51.2	50	0.3	280	74.6	41.8	38	52.7
1066	2086	16549	16567	54.9	52.6	4586	17041	17022	54.1	50	0.8	493	75.8	42.2	38	54.4
1067	2087	20138	20158	50.1	42.9	4587	20615	20597	50.6	47.4	0.5	478	75	40.4	38	52.7
1068	2088	20078	20099	50.5	40.9	4588	20615	20597	50.6	47.4	0.1	538	75.3	40.5	38	52.9
1069	2089	13039	13057	51.1	52.6	4589	13325	13305	50.5	47.6	0.6	287	75.8	44.6	38	53.3
1070	2090	16549	16567	54.9	52.6	4590	17041	17023	53.5	52.6	1.4	493	75.8	42.2	38	54.2
1071	2091	13701	13725	53.6	40	4591	14124	14106	52.4	52.6	1.2	424	75.1	41	38	53.4
1072	2092	12975	12993	51.4	47.4	4592	13320	13300	51.4	47.6	0	346	76.1	44.2	38	53.8
1073	2093	16548	16566	54.9	52.6	4593	16779	16758	53.5	50	1.4	232	74	41.4	38	52.9
1074	2094	3361	3381	50.5	42.9	4594	3500	3481	51.2	50	0.7	140	74.1	46.4	38	52.1
1075	2095	3361	3381	50.5	42.9	4595	3503	3484	51.5	50	1	143	74.4	46.9	38	52.3
1076	2096	16368	16387	50.2	45	4596	16780	16760	51.4	42.9	1.2	413	74.9	40.7	38	52.6
1077	2097	7725	7742	50	50	4597	8188	8168	50.4	42.9	0.3	464	75.6	41.8	38	53
1078	2098	8867	8887	52.3	47.6	4598	9597	9573	53.4	40	1.1	731	75.9	41.2	38	53.9
1079	2099	2223	2244	51.4	45.5	4599	2672	2654	50.9	52.6	0.5	450	77	45.3	38	54.3
1080	2100	10242	10265	51.2	41.7	4600	10605	10588	51.1	50	0.2	364	74.5	40.1	38	52.6
1081	2101	8867	8888	52.7	45.5	4601	9253	9235	51.6	47.4	1.1	387	75.1	41.3	38	53.2
1082	2102	3361	3381	50.5	42.9	4602	3504	3485	50.4	45	0.1	144	74.3	46.5	38	52.2
1083	2103	98	118	50.6	42.9	4603	314	296	50.6	47.4	0	217	75.9	46.5	38	53.4
1084	2104	12233	12251	51.1	52.6	4604	12498	12480	50	47.4	1.1	266	74.8	42.5	38	52.5
1085	2105	9926	9944	50.5	52.6	4605	10455	10434	51.1	40.9	0.6	530	75.3	40.6	38	52.9
1086	2106	3360	3380	51.4	42.9	4606	3497	3478	51.3	50	0.1	138	74	46.4	38	52.3
1087	2107	9926	9944	50.5	52.6	4607	10455	10435	50.5	42.9	0	530	75.3	40.6	38	52.9

1088	2108	10140	10159	52.4	50	4608	10608	10589	51	50	1.4	469	75	40.3	38	52.9
1089	2109	9931	9950	50.2	45	4609	10455	10435	50.5	42.9	0.3	525	75.3	40.6	38	52.8
1090	2110	3219	3238	50.7	50	4610	3500	3481	51.2	50	0.5	282	74.7	41.8	38	52.6
1091	2111	3219	3238	50.7	50	4611	3497	3478	51.3	50	0.6	279	74.7	41.9	38	52.6
1092	2112	3360	3380	51.4	42.9	4612	3500	3481	51.2	50	0.3	141	74	46.1	38	52.3
1093	2113	3360	3380	51.4	42.9	4613	3503	3484	51.5	50	0	144	74.3	46.5	38	52.5
1094	2114	2223	2244	51.4	45.5	4614	2672	2653	51.6	50	0.2	450	77	45.3	38	54.4
1095	2115	9922	9941	51.3	50	4615	10449	10428	51.9	40.9	0.7	528	75.4	40.9	38	53.3
1096	2116	13039	13057	51.1	52.6	4616	13312	13294	51	52.6	0.1	274	75.7	44.5	38	53.4
1097	2117	15951	15973	52.1	43.5	4617	16174	16154	50.4	42.9	1.7	224	73.5	40.6	38	51.7
1098	2118	13176	13196	51.4	47.6	4618	13545	13526	52.9	55	1.5	370	77	46.2	38	54.4
1099	2119	11541	11562	51.5	40.9	4619	11983	11965	53	52.6	1.5	443	75	40.6	38	53.1
1100	2120	2429	2447	50.2	47.4	4620	3056	3038	50.8	52.6	0.6	628	76.3	42.7	38	53.6
1101	2121	11545	11563	50.8	47.4	4621	12258	12238	50.3	42.9	0.5	714	76.2	42	38	53.5
1102	2122	8868	8889	50.4	40.9	4622	9245	9226	50	45	0.4	378	74.9	41	38	52.6
1103	2123	27361	27380	52.4	55	4623	27466	27448	52.3	52.6	0.1	106	72.5	46.2	38	51.5
1104	2124	8861	8880	50.2	45	4624	9340	9319	50.8	45.5	0.6	480	75.5	41.5	38	53
1105	2125	1784	1802	51.8	52.6	4625	2113	2094	50.1	45	1.7	330	76	44.2	38	53.3
1106	2126	8868	8889	50.4	40.9	4626	9107	9086	51.6	45.5	1.2	240	74	41.2	38	52
1107	2127	19795	19814	50.4	45	4627	20099	20078	50.5	40.9	0	305	74.4	40.7	38	52.3
1108	2128	26708	26731	54.2	41.7	4628	27347	27324	52.3	41.7	1.9	640	75.6	40.8	38	53.7
1109	2129	19794	19813	50	50	4629	19920	19899	50.2	40.9	0.2	127	72.3	43.3	38	50.7
1110	2130	3031	3051	51.3	52.4	4630	3650	3631	53.1	50	1.8	620	76.5	43.1	38	54
1111	2131	3031	3051	51.3	52.4	4631	3647	3628	50.6	45	0.7	617	76.4	42.9	38	53.8
1112	2132	19794	19813	50	50	4632	19922	19902	50	42.9	0	129	72.5	43.4	38	50.8
1113	2133	12236	12256	51.2	42.9	4633	12994	12976	50.3	47.4	0.8	759	76.4	42.4	38	53.7
1114	2134	26708	26731	54.2	41.7	4634	27467	27449	52.8	47.4	1.4	760	76	41.3	38	54.1
1115	2135	19716	19737	52.2	45.5	4635	19922	19901	51.5	45.5	0.7	207	73.5	41.1	38	52
1116	2136	19715	19735	52.5	47.6	4636	19922	19901	51.5	45.5	0.9	208	73.6	41.3	38	52.1
1117	2137	3360	3379	50.7	45	4637	3503	3484	51.5	50	0.7	144	74.3	46.5	38	52.3
1118	2138	9055	9079	52.8	40	4638	9364	9346	53.9	52.6	1.1	310	75.3	42.9	38	53.7
1119	2139	1782	1801	52.7	50	4639	1881	1861	54.5	52.4	1.8	100	72.4	47	38	51.6
1120	2140	26708	26727	50	45	4640	27468	27450	51.9	47.4	1.8	761	75.9	41.3	38	53.3
1121	2141	26708	26727	50	45	4641	27468	27451	51.1	50	1.1	761	75.9	41.3	38	53.3
1122	2142	4593	4613	51.5	47.6	4642	4995	4975	51.7	42.9	0.1	403	76	43.4	38	53.8
1123	2143	19709	19730	51.3	40.9	4643	19930	19911	50.7	50	0.5	222	74	41.9	38	52.1
1124	2144	26421	26441	51.5	42.9	4644	26587	26570	50.2	50	1.3	167	72.3	40.1	38	50.8
1125	2145	18979	19000	51.6	45.5	4645	19217	19195	51.7	43.5	0	239	73.5	40.2	38	52.1
1126	2146	18703	18724	53.5	50	4646	19476	19453	53.5	41.7	0	774	75.6	40.3	38	54
1127	2147	4255	4276	51.7	45.5	4647	4708	4690	50.3	47.4	1.4	454	75.1	40.7	38	52.8
1128	2148	3232	3252	51.1	47.6	4648	3503	3484	51.5	50	0.4	272	74.6	41.9	38	52.6
1129	2149	26421	26441	51.5	42.9	4649	26656	26636	51.3	47.6	0.2	236	74.2	41.9	38	52.5
1130	2150	3232	3252	51.1	47.6	4650	3504	3485	50.4	45	0.7	273	74.6	41.8	38	52.4
1131	2151	26421	26441	51.5	42.9	4651	26660	26641	50.2	50	1.3	240	74.2	41.7	38	52.1
1132	2152	26421	26441	51.5	42.9	4652	26683	26665	52.7	52.6	1.2	263	74.8	42.6	38	52.9
1133	2153	26421	26441	51.5	42.9	4653	26686	26669	50.5	50	0.9	266	74.8	42.5	38	52.6
1134	2154	26421	26441	51.5	42.9	4654	26691	26673	51.3	47.4	0.1	271	74.8	42.4	38	52.9
1135	2155	18704	18724	50.8	47.6	4655	19476	19456	50.5	42.9	0.3	773	75.5	40.2	38	53.1
1136	2156	18704	18724	50.8	47.6	4656	19482	19463	50.1	45	0.7	779	75.5	40.2	38	53
1137	2157	942	960	52.1	52.6	4657	1498	1481	51	50	1.1	557	76.9	44.5	38	54.3
1138	2158	942	960	52.1	52.6	4658	1497	1480	50.3	50	1.9	556	77	44.6	38	54.1

1139	2159	13040	13059	50.9	50	4659	13312	13294	51	52.6	0.1	273	75.6	44.3	38	53.3
1140	2160	18696	18715	51.7	50	4660	19476	19453	53.5	41.7	1.8	781	75.6	40.3	38	53.5
1141	2161	18696	18715	51.7	50	4661	19476	19456	50.5	42.9	1.3	781	75.6	40.3	38	53.1
1142	2162	3232	3251	50.3	50	4662	3503	3484	51.5	50	1.1	272	74.6	41.9	38	52.4
1143	2163	3031	3051	51.3	52.4	4663	3646	3625	52	40.9	0.7	616	76.4	42.9	38	54
1144	2164	9130	9150	51.3	42.9	4664	9560	9541	50.9	45	0.4	431	75.3	41.3	38	53
1145	2165	18224	18243	53.1	50	4665	18696	18672	53.9	40	0.8	473	75.7	42.1	38	54
1146	2166	18224	18243	53.1	50	4666	18696	18673	53.4	41.7	0.3	473	75.7	42.1	38	54
1147	2167	18225	18243	51.4	52.6	4667	18697	18679	51.9	52.6	0.5	473	75.8	42.3	38	53.6
1148	2168	9130	9150	51.3	42.9	4668	9560	9540	51.6	42.9	0.3	431	75.3	41.3	38	53.2
1149	2169	8866	8885	51.1	45	4669	9252	9235	50.1	50	1	387	75.1	41.3	38	52.7
1150	2170	9130	9150	51.3	42.9	4670	9559	9539	50.6	42.9	0.7	430	75.3	41.4	38	53
1151	2171	12267	12290	54.5	41.7	4671	12501	12480	53.5	45.5	1	235	74.3	42.1	38	53.2
1152	2172	3427	3446	52.7	50	4672	3650	3631	53.1	50	0.4	224	74.3	42.4	38	52.9
1153	2173	3427	3446	52.7	50	4673	3648	3628	52.3	42.9	0.4	222	74	41.9	38	52.6
1154	2174	26039	26058	54	55	4674	26184	26164	52.4	42.9	1.6	146	71.8	40.4	38	51.1
1155	2175	3230	3249	50.1	45	4675	3503	3484	51.5	50	1.4	274	74.5	41.6	38	52.3
1156	2176	3230	3249	50.1	45	4676	3504	3485	50.4	45	0.3	275	74.4	41.5	38	52.2
1157	2177	3427	3446	52.7	50	4677	3646	3625	52	40.9	0.6	220	74	41.8	38	52.5
1158	2178	3429	3449	50.4	42.9	4678	3647	3628	50.6	45	0.2	219	73.9	41.6	38	51.9
1159	2179	3429	3449	50.4	42.9	4679	3646	3625	52	40.9	1.6	218	73.7	41.3	38	51.8
1160	2180	8866	8885	51.1	45	4680	9249	9231	50.8	47.4	0.3	384	75.1	41.4	38	52.9
1161	2181	3428	3449	52.8	45.5	4681	3650	3631	53.1	50	0.3	223	74.2	42.2	38	52.8
1162	2182	18077	18098	52.9	50	4682	18696	18672	53.9	40	1	620	76.2	42.4	38	54.3
1163	2183	18078	18098	51.5	47.6	4683	18696	18673	53.4	41.7	1.9	619	76.2	42.3	38	53.9
1164	2184	8866	8885	51.1	45	4684	9249	9230	51.5	45	0.4	384	75.1	41.4	38	53
1165	2185	3229	3248	50.6	50	4685	3503	3484	51.5	50	0.8	275	74.6	41.8	38	52.5
1166	2186	12267	12290	54.5	41.7	4686	12495	12476	52.7	45	1.9	229	74.1	41.9	38	52.8
1167	2187	8220	8240	54	47.6	4687	8929	8910	54.5	55	0.4	710	75.4	40	38	54.1
1168	2188	18080	18098	51.2	52.6	4688	18238	18219	50.3	45	0.9	159	74	44.7	38	52
1169	2189	18080	18098	51.2	52.6	4689	18239	18220	50	45	1.2	160	73.9	44.4	38	51.8
1170	2190	18080	18098	51.2	52.6	4690	18697	18679	51.9	52.6	0.7	618	76.3	42.6	38	53.8
1171	2191	18076	18097	53.1	45.5	4691	18712	18693	54.8	55	1.7	637	76.3	42.5	38	54.4
1172	2192	8866	8885	51.1	45	4692	9245	9226	50	45	1.1	380	75	41.1	38	52.6
1173	2193	943	961	50.3	47.4	4693	1498	1481	51	50	0.8	556	76.9	44.4	38	54
1174	2194	18075	18095	50.6	47.6	4694	18642	18622	50.5	42.9	0.1	568	76.2	42.6	38	53.6
1175	2195	18075	18095	50.6	47.6	4695	18662	18641	50.4	40.9	0.2	588	76.3	42.7	38	53.6
1176	2196	8866	8885	51.1	45	4696	9107	9086	51.6	45.5	0.5	242	74.1	41.3	38	52.3
1177	2197	943	961	50.3	47.4	4697	1497	1480	50.3	50	0	555	76.9	44.5	38	54
1178	2198	7400	7417	50.2	50	4698	8190	8172	50.3	47.4	0.1	791	76.4	42.2	38	53.6
1179	2199	13039	13058	51.8	50	4699	13314	13297	51	50	0.9	276	75.7	44.6	38	53.4
1180	2200	7725	7743	50.8	47.4	4700	8187	8167	50.4	42.9	0.5	463	75.6	41.9	38	53.1
1181	2201	18074	18094	51.1	42.9	4701	18642	18622	50.5	42.9	0.5	569	76.2	42.5	38	53.6
1182	2202	25782	25805	52.1	41.7	4702	26174	26153	51	40.9	1.1	393	74.8	40.5	38	52.8
1183	2203	9131	9151	50.4	42.9	4703	9560	9541	50.9	45	0.5	430	75.3	41.4	38	52.9
1184	2204	25782	25805	52.1	41.7	4704	26183	26162	52.8	45.5	0.7	402	74.7	40.3	38	53.1
1185	2205	9131	9151	50.4	42.9	4705	9560	9540	51.6	42.9	1.2	430	75.3	41.4	38	52.9
1186	2206	7725	7743	50.8	47.4	4706	8188	8168	50.4	42.9	0.5	464	75.6	41.8	38	53.1
1187	2207	985	1004	51.1	50	4707	1494	1476	50.7	47.4	0.4	510	76.5	43.7	38	53.9
1188	2208	13039	13058	51.8	50	4708	13323	13304	51.1	45	0.7	285	75.8	44.6	38	53.5
1189	2209	9131	9151	50.4	42.9	4709	9559	9539	50.6	42.9	0.3	429	75.3	41.5	38	52.9

1190	2210	12352	12375	52.9	41.7	4710	12499	12480	51.8	45	1.1	148	73.3	43.9	38	52
1191	2211	3225	3244	52.4	55	4711	3646	3625	52	40.9	0.4	422	75.4	41.7	38	53.5
1192	2212	25676	25697	51.9	40.9	4712	25784	25765	53.3	50	1.4	109	70.3	40.4	38	49.9
1193	2213	25363	25381	51.1	52.6	4713	25548	25531	51.1	50	0	186	73.7	42.5	38	52
1194	2214	25363	25381	51.1	52.6	4714	25645	25626	50.8	45	0.4	283	74.2	40.6	38	52.3
1195	2215	18074	18093	50.3	45	4715	18642	18622	50.5	42.9	0.2	569	76.2	42.5	38	53.5
1196	2216	3225	3244	52.4	55	4716	3647	3628	50.6	45	1.8	423	75.5	41.8	38	53.1
1197	2217	3225	3244	52.4	55	4717	3650	3631	53.1	50	0.7	426	75.5	42	38	53.7
1198	2218	12352	12375	52.9	41.7	4718	12494	12476	52.2	47.4	0.6	143	73.2	44.1	38	52
1199	2219	13039	13058	51.8	50	4719	13326	13306	50.7	42.9	1.2	288	75.8	44.4	38	53.4
1200	2220	7617	7636	50.9	50	4720	8188	8169	50.5	45	0.5	572	76.1	42.3	38	53.5
1201	2221	988	1006	52.2	52.6	4721	1697	1678	50.3	45	2	710	76.9	43.8	38	54
1202	2222	12232	12250	51.9	52.6	4722	12739	12719	50.3	42.9	1.7	508	75.8	42.1	38	53.3
1203	2223	12232	12250	51.9	52.6	4723	12739	12718	51	40.9	1	508	75.8	42.1	38	53.5
1204	2224	988	1006	52.2	52.6	4724	1697	1677	51	42.9	1.2	710	76.9	43.8	38	54.2
1205	2225	8867	8886	50.7	50	4725	9341	9322	51.1	50	0.5	475	75.7	41.9	38	53.3
1206	2226	3223	3242	51.8	55	4726	3650	3631	53.1	50	1.3	428	75.6	42.1	38	53.6
1207	2227	8867	8886	50.7	50	4727	9340	9319	50.8	45.5	0.1	474	75.6	41.8	38	53.2
1208	2228	988	1006	52.2	52.6	4728	1697	1676	51.7	40.9	0.6	710	76.9	43.8	38	54.4
1209	2229	988	1006	52.2	52.6	4729	1694	1673	51.7	40.9	0.5	707	76.9	43.8	38	54.5
1210	2230	988	1006	52.2	52.6	4730	1494	1476	50.7	47.4	1.5	507	76.5	43.8	38	53.9
1211	2231	9931	9950	50.2	45	4731	10455	10434	51.1	40.9	1	525	75.3	40.6	38	52.8
1212	2232	3224	3242	50.5	52.6	4732	3646	3625	52	40.9	1.5	423	75.4	41.6	38	53
1213	2233	3224	3242	50.5	52.6	4733	3647	3628	50.6	45	0.1	424	75.4	41.7	38	53.1
1214	2234	3016	3036	50.2	42.9	4734	3187	3166	50.3	45.5	0.1	172	74.6	45.3	38	52.4
1215	2235	24559	24579	52	52.4	4735	25182	25164	51.4	47.4	0.6	624	76	41.8	38	53.7
1216	2236	1782	1802	53.3	47.6	4736	1881	1861	54.5	52.4	1.2	100	72.4	47	38	51.8
1217	2237	7880	7900	50.3	42.9	4737	8188	8169	50.5	45	0.1	309	74.8	41.7	38	52.6
1218	2238	8861	8880	50.2	45	4738	9248	9229	50.1	45	0	388	75	41	38	52.6
1219	2239	8868	8889	50.4	40.9	4739	9312	9293	50.6	45	0.1	445	75.3	41.3	38	53
1220	2240	17790	17813	54.3	41.7	4740	18220	18201	56.1	55	1.8	431	74.9	40.4	38	53.8
1221	2241	24569	24590	56.6	54.5	4741	25184	25164	55.9	52.4	0.7	616	75.9	41.7	38	55
1222	2242	13176	13196	51.4	47.6	4742	13328	13307	51.2	45.5	0.2	153	73.4	43.8	38	51.9
1223	2243	8861	8880	50.2	45	4743	9254	9236	50.6	47.4	0.4	394	74.9	40.9	38	52.6
1224	2244	24622	24643	57.1	54.5	4744	25400	25377	57.2	50	0.1	779	75.7	40.7	38	55.2
1225	2245	8868	8889	50.4	40.9	4745	9256	9237	50.8	45	0.4	389	75	41.1	38	52.7
1226	2246	3361	3381	50.5	42.9	4746	3497	3478	51.3	50	0.8	137	74.1	46.7	38	52.1
1227	2247	4593	4613	51.5	47.6	4747	4711	4693	50.4	47.4	1.1	119	71.5	42	38	50.2
1228	2248	19911	19930	50.7	50	4748	20615	20597	50.6	47.4	0.1	705	75.5	40.3	38	53.1
1229	2249	3221	3239	51.5	52.6	4749	3497	3478	51.3	50	0.2	277	74.6	41.9	38	52.7
1230	2250	3223	3241	50.2	52.6	4750	3504	3485	50.4	45	0.2	282	74.8	42.2	38	52.5
1231	2251	3223	3241	50.2	52.6	4751	3503	3484	51.5	50	1.2	281	74.9	42.3	38	52.6
1232	2252	3360	3380	51.4	42.9	4752	3504	3485	50.4	45	1	145	74.2	46.2	38	52.2
1233	2253	4593	4613	51.5	47.6	4753	4711	4692	51.2	45	0.3	119	71.5	42	38	50.5
1234	2254	4593	4613	51.5	47.6	4754	4710	4691	50.2	45	1.4	118	71.6	42.4	38	50.2
1235	2255	3016	3036	50.2	42.9	4755	3186	3165	50.4	40.9	0.2	171	74.4	45	38	52.3
1236	2256	29182	29206	55.4	44	4756	29301	29282	55.3	55	0.1	120	73.4	46.7	38	53.1
1237	2257	29183	29206	52.9	41.7	4757	29306	29287	54.6	55	1.7	124	73.3	46	38	52.3
1238	2258	29186	29206	51.3	42.9	4758	29298	29279	52.6	55	1.3	113	72.8	46	38	51.5
1239	2259	16979	17000	52.6	50	4759	17483	17465	54.4	52.6	1.8	505	75.9	42.2	38	54
1240	2260	29182	29205	54.6	41.7	4760	29298	29279	52.6	55	1.9	117	73.1	46.2	38	52

1241	2261	16981	17000	51.3	50	4761	17111	17090	51.1	40.9	0.2	131	74.5	48.1	38	52.6
1242	2262	13177	13197	50.3	42.9	4762	13949	13932	51.6	50	1.3	773	75.8	41	38	53.3
1243	2263	8867	8887	52.3	47.6	4763	9252	9234	51.4	52.6	0.9	386	75.1	41.5	38	53.1
1244	2264	24420	24440	50.8	42.9	4764	25081	25063	52.4	52.6	1.6	662	75.7	40.9	38	53.3
1245	2265	7727	7745	50.8	47.4	4765	8188	8169	50.5	45	0.4	462	75.6	41.8	38	53.1
1246	2266	2387	2405	51.6	52.6	4766	3055	3036	50.6	50	1.1	669	76.7	43.3	38	53.9
1247	2267	2671	2692	52.1	40.9	4767	3055	3036	50.6	50	1.5	385	74.8	40.5	38	52.6
1248	2268	29182	29202	51.2	42.9	4768	29298	29279	52.6	55	1.4	117	73.1	46.2	38	51.6
1249	2269	24418	24439	52.9	45.5	4769	25081	25063	52.4	52.6	0.5	664	75.7	41.1	38	53.8
1250	2270	12373	12391	50.8	47.4	4770	12992	12974	51.2	52.6	0.4	620	76.5	43.1	38	53.9
1251	2271	29179	29199	51.4	42.9	4771	29298	29279	52.6	55	1.2	120	73.4	46.7	38	51.9
1252	2272	1783	1803	54.2	47.6	4772	1882	1861	56	50	1.8	100	72.4	47	38	52
1253	2273	12373	12391	50.8	47.4	4773	12498	12480	50	47.4	0.7	126	72.8	44.4	38	51
1254	2274	7728	7746	51.7	52.6	4774	8190	8172	50.3	47.4	1.4	463	75.6	41.9	38	53.1
1255	2275	16875	16896	52.2	45.5	4775	17064	17045	51.4	50	0.8	190	74.5	44.2	38	52.7
1256	2276	1402	1425	52.8	41.7	4776	2103	2082	52	45.5	0.8	702	76.7	43.3	38	54.4
1257	2277	28971	28993	51.9	43.5	4777	29358	29339	52.8	50	0.9	388	76.3	44.3	38	54.1
1258	2278	24380	24399	55	55	4778	25080	25061	54.1	50	1	701	75.9	41.4	38	54.5
1259	2279	24380	24399	55	55	4779	25080	25062	53.5	52.6	1.6	701	75.9	41.4	38	54.3
1260	2280	3168	3189	51	45.5	4780	3497	3478	51.3	50	0.3	330	75.3	42.4	38	53.1
1261	2281	1402	1426	54.1	40	4781	1626	1602	56.1	44	1.9	225	76.4	47.6	38	54.8
1262	2282	24379	24398	55	55	4782	25080	25061	54.1	50	1	702	75.9	41.3	38	54.4
1263	2283	24379	24398	55	55	4783	25080	25062	53.5	52.6	1.6	702	75.9	41.3	38	54.3
1264	2284	16875	16895	51.6	47.6	4784	17062	17045	50.2	50	1.4	188	74.4	44.1	38	52.2
1265	2285	12726	12746	51.3	47.6	4785	12998	12979	50.1	45	1.2	273	75.2	43.2	38	52.7
1266	2286	24378	24397	55	55	4786	24517	24494	53.2	41.7	1.8	140	72.7	42.9	38	51.9
1267	2287	24378	24397	55	55	4787	25080	25061	54.1	50	1	703	75.9	41.3	38	54.4
1268	2288	28939	28961	55.2	47.8	4788	29306	29285	56.7	54.5	1.5	368	76.6	45.1	38	55.3
1269	2289	28940	28961	53.1	45.5	4789	29306	29287	54.6	55	1.5	367	76.5	45	38	54.6
1270	2290	28941	28961	51.6	42.9	4790	29298	29279	52.6	55	1	358	76.3	44.7	38	54
1271	2291	28178	28200	52	43.5	4791	28284	28265	52.9	50	0.9	107	74.7	51.4	38	53
1272	2292	28941	28961	51.6	42.9	4792	29358	29339	52.8	50	1.2	418	76.5	44.5	38	54.2
1273	2293	24378	24397	55	55	4793	25080	25062	53.5	52.6	1.6	703	75.9	41.3	38	54.2
1274	2294	28938	28960	56.1	47.8	4794	29306	29285	56.7	54.5	0.6	369	76.5	45	38	55.5
1275	2295	12234	12252	50.6	47.4	4795	12498	12480	50	47.4	0.5	265	74.7	42.3	38	52.4
1276	2296	28939	28960	54.7	50	4796	29306	29287	54.6	55	0.1	368	76.6	45.1	38	55.1
1277	2297	28140	28158	54.1	52.6	4797	28411	28393	52.9	52.6	1.1	272	78.8	52.2	38	56.2
1278	2298	28941	28960	50.9	45	4798	29298	29279	52.6	55	1.7	358	76.3	44.7	38	53.8
1279	2299	28140	28158	54.1	52.6	4799	28416	28396	52.4	47.6	1.7	277	78.8	52	38	56
1280	2300	28941	28960	50.9	45	4800	29358	29339	52.8	50	1.9	418	76.5	44.5	38	53.9
1281	2301	24179	24200	53.3	40.9	4801	24815	24791	54.5	40	1.2	637	75.8	41.3	38	54.1
1282	2302	28938	28956	50.8	47.4	4802	29298	29279	52.6	55	1.8	361	76.4	44.9	38	53.8
1283	2303	12726	12746	51.3	47.6	4803	12992	12974	51.2	52.6	0.1	267	75.2	43.4	38	53.1
1284	2304	16874	16893	52.1	50	4804	17062	17045	50.2	50	1.9	189	74.6	44.4	38	52.3
1285	2305	1352	1371	56.1	55	4805	1484	1464	54.3	47.6	1.8	133	74.9	48.9	38	53.8
1286	2306	11540	11561	53.8	45.5	4806	11983	11965	53	52.6	0.7	444	75.1	40.8	38	53.6
1287	2307	24179	24199	52.7	42.9	4807	24815	24792	53.4	41.7	0.7	637	75.8	41.3	38	53.9
1288	2308	16555	16572	50.3	50	4808	16777	16758	51.5	50	1.2	223	73.6	40.8	38	51.7
1289	2309	24178	24198	52.7	42.9	4809	24815	24791	54.5	40	1.8	638	75.7	41.2	38	53.9
1290	2310	3192	3213	51.8	45.5	4810	3650	3631	53.1	50	1.3	459	75.7	42	38	53.6
1291	2311	3192	3213	51.8	45.5	4811	3647	3628	50.6	45	1.2	456	75.6	41.9	38	53.2

1292	2312	24174	24195	52.5	40.9	4812	24815	24792	53.4	41.7	0.9	642	75.8	41.3	38	53.9
1293	2313	16553	16571	53.4	52.6	4813	16780	16760	51.4	42.9	2	228	73.7	40.8	38	52.1
1294	2314	16550	16568	54.1	52.6	4814	17041	17023	53.5	52.6	0.6	492	75.9	42.3	38	54.3
1295	2315	3192	3213	51.8	45.5	4815	3646	3625	52	40.9	0.2	455	75.5	41.8	38	53.5
1296	2316	16551	16568	51.1	50	4816	16777	16758	51.5	50	0.4	227	73.9	41.4	38	52.2
1297	2317	12373	12391	50.8	47.4	4817	12998	12979	50.1	45	0.7	626	76.4	43	38	53.6
1298	2318	28868	28887	50.7	45	4818	29414	29395	50.5	50	0.2	547	77	44.8	38	54.2
1299	2319	24028	24047	53.8	50	4819	24815	24791	54.5	40	0.7	788	76.3	42	38	54.6
1300	2320	2427	2445	52.1	52.6	4820	3056	3038	50.8	52.6	1.3	630	76.4	42.9	38	53.8
1301	2321	28867	28886	53.2	50	4821	29306	29288	53.5	52.6	0.3	440	76.9	45.2	38	54.9
1302	2322	24021	24044	52.8	41.7	4822	24815	24791	54.5	40	1.6	795	76.2	41.9	38	54.3
1303	2323	28867	28885	51.5	52.6	4823	29414	29395	50.5	50	0.9	548	77.1	44.9	38	54.2
1304	2324	12369	12388	50.6	45	4824	13155	13137	52.1	52.6	1.5	787	76.8	43.3	38	54
1305	2325	27368	27392	58.2	48	4825	27467	27443	59.4	48	1.2	100	71.2	44	38	52.4
1306	2326	27369	27392	57.2	50	4826	27468	27444	58.4	44	1.2	100	71.2	44	38	52.1
1307	2327	27369	27392	57.2	50	4827	27468	27445	58.1	45.8	0.8	100	71.2	44	38	52.1
1308	2328	23841	23863	53.7	47.8	4828	24022	24003	55.5	55	1.7	182	74.4	44.5	38	53.3
1309	2329	3192	3213	51.8	45.5	4829	3497	3478	51.3	50	0.5	306	75	42.2	38	53
1310	2330	23843	23863	50.3	42.9	4830	24526	24506	50.3	42.9	0	684	76.1	41.8	38	53.4
1311	2331	27366	27389	56.1	45.8	4831	27465	27443	56.4	47.8	0.3	100	71.2	44	38	51.8
1312	2332	27366	27389	56.1	45.8	4832	27465	27444	55.6	45.5	0.6	100	71.2	44	38	51.6
1313	2333	27366	27389	56.1	45.8	4833	27465	27445	55.1	47.6	1	100	71.2	44	38	51.5
1314	2334	16549	16567	54.9	52.6	4834	16779	16758	53.5	50	1.4	231	74	41.6	38	53
1315	2335	27369	27389	52.5	47.6	4835	27468	27448	53.7	47.6	1.1	100	71.2	44	38	50.7
1316	2336	27369	27389	52.5	47.6	4836	27468	27449	52.6	45	0	100	71.2	44	38	50.7
1317	2337	27369	27389	52.5	47.6	4837	27468	27450	51.9	47.4	0.7	100	71.2	44	38	50.5
1318	2338	28654	28672	50.6	52.6	4838	29412	29393	50.3	45	0.2	759	77.9	46.1	38	54.7
1319	2339	2429	2447	50.2	47.4	4839	3053	3034	50.3	50	0.1	625	76.3	42.6	39	53.6
1320	2340	1442	1461	51.6	55	4840	1697	1676	51.7	40.9	0	256	75.8	45.3	39	53.7
1321	2341	1442	1461	51.6	55	4841	1697	1677	51	42.9	0.6	256	75.8	45.3	39	53.5
1322	2342	1442	1461	51.6	55	4842	1697	1678	50.3	45	1.3	256	75.8	45.3	39	53.3
1323	2343	3214	3233	51.1	50	4843	3504	3485	50.4	45	0.7	291	74.8	41.9	39	52.6
1324	2344	3214	3233	51.1	50	4844	3503	3484	51.5	50	0.4	290	74.8	42.1	39	52.8
1325	2345	27374	27392	50.6	47.4	4845	27674	27653	52.5	40.9	1.9	301	74.1	40.2	39	52.2
1326	2346	9930	9949	52.2	50	4846	10670	10649	51.3	40.9	0.9	741	75.8	40.9	39	53.5
1327	2347	1442	1461	51.6	55	4847	2103	2083	50.6	42.9	1	662	76.7	43.4	39	53.9
1328	2348	8867	8887	52.3	47.6	4848	9375	9354	50.4	40.9	2	509	75.7	41.8	39	53.2
1329	2349	16367	16386	51.4	50	4849	16775	16755	51.1	42.9	0.3	409	75	40.8	39	52.9
1330	2350	18081	18100	51.7	50	4850	18702	18685	50.2	50	1.5	622	76.2	42.4	39	53.5
1331	2351	18083	18102	50.6	45	4851	18702	18685	50.2	50	0.4	620	76.1	42.3	39	53.4
1332	2352	18094	18113	51	50	4852	18702	18685	50.2	50	0.8	609	76.1	42.2	39	53.4
1333	2353	8865	8884	50.4	45	4853	9254	9236	50.6	47.4	0.2	390	75	41	39	52.7
1334	2354	16367	16386	51.4	50	4854	16774	16754	50.4	42.9	1	408	75	40.9	39	52.7
1335	2355	18008	18028	53	52.4	4855	18220	18202	54.8	52.6	1.9	213	74.4	43.2	39	53.1
1336	2356	27369	27389	52.5	47.6	4856	27674	27653	52.5	40.9	0.1	306	74.3	40.5	39	52.9
1337	2357	16367	16386	51.4	50	4857	16774	16753	51.1	40.9	0.3	408	75	40.9	39	52.9
1338	2358	1442	1461	51.6	55	4858	2113	2094	50.1	45	1.5	672	76.7	43.3	39	53.8
1339	2359	7876	7895	51.5	45	4859	8190	8172	50.3	47.4	1.2	315	75.1	42.2	39	52.7
1340	2360	18696	18715	51.7	50	4860	19482	19463	50.1	45	1.7	787	75.6	40.3	39	53
1341	2361	12370	12388	50.1	47.4	4861	12911	12892	50.5	50	0.4	542	76.1	42.4	39	53.4
1342	2362	887	905	50.1	47.4	4862	1493	1473	52	47.6	1.9	607	77.1	44.6	39	54.1

1343	2363	16367	16387	51.8	47.6	4863	16774	16751	53.6	41.7	1.8	408	75	40.9	39	53.2
1344	2364	16378	16397	50.4	45	4864	17111	17090	51.1	40.9	0.7	734	76.3	42.2	39	53.6
1345	2365	16378	16397	50.4	45	4865	16781	16761	51.3	47.6	0.8	404	75.1	41.1	39	52.8
1346	2366	1402	1425	52.8	41.7	4866	1501	1478	54.6	41.7	1.8	100	72	46	39	51.3
1347	2367	16378	16397	50.4	45	4867	16777	16758	51.5	50	1	400	75	41	39	52.7
1348	2368	16378	16397	50.4	45	4868	16775	16756	50.3	45	0.1	398	75	41	39	52.7
1349	2369	16378	16397	50.4	45	4869	16775	16755	51.1	42.9	0.6	398	75	41	39	52.7
1350	2370	16378	16397	50.4	45	4870	16774	16754	50.4	42.9	0	397	75	41.1	39	52.7
1351	2371	16378	16397	50.4	45	4871	16774	16753	51.1	40.9	0.7	397	75	41.1	39	52.8
1352	2372	16378	16397	50.4	45	4872	16774	16752	52.2	43.5	1.8	397	75	41.1	39	52.8
1353	2373	10250	10274	51.6	40	4873	10608	10589	51	50	0.6	359	74.6	40.4	39	52.6
1354	2374	16548	16566	54.9	52.6	4874	17112	17090	53.3	43.5	1.6	565	76.3	42.8	39	54.5
1355	2375	19709	19730	51.3	40.9	4875	19922	19902	50	42.9	1.2	214	73.8	41.6	39	51.8
1356	2376	3218	3237	50.5	45	4876	3504	3485	50.4	45	0.1	287	74.7	41.8	39	52.5
1357	2377	3218	3237	50.5	45	4877	3503	3484	51.5	50	0.9	286	74.7	42	39	52.6
1358	2378	19709	19730	51.3	40.9	4878	19920	19899	50.2	40.9	1.1	212	73.7	41.5	39	51.8
1359	2379	1402	1422	50.2	42.9	4879	1501	1480	51.9	40.9	1.7	100	72	46	39	50.6
1360	2380	1402	1422	50.2	42.9	4880	1501	1481	51.2	42.9	1.1	100	72	46	39	50.6
1361	2381	8867	8886	50.7	50	4881	9249	9230	51.5	45	0.9	383	75.2	41.5	39	52.9
1362	2382	19794	19813	50	50	4882	19928	19908	52	52.4	2	135	72.8	43.7	39	51.1
1363	2383	8867	8886	50.7	50	4883	9249	9231	50.8	47.4	0.2	383	75.2	41.5	39	52.9
1364	2384	9927	9945	50.8	52.6	4884	10183	10165	51.7	47.4	0.9	257	75.3	44	39	53.1
1365	2385	27366	27384	52.2	52.6	4885	27566	27546	50.7	47.6	1.5	201	74.7	44.3	39	52.6
1366	2386	9927	9945	50.8	52.6	4886	10183	10166	50.9	50	0.1	257	75.3	44	39	53.1
1367	2387	27366	27384	52.2	52.6	4887	27568	27548	50.2	42.9	1.9	203	74.6	43.8	39	52.4
1368	2388	27366	27384	52.2	52.6	4888	27571	27551	51.4	42.9	0.8	206	74.5	43.7	39	52.7
1369	2389	887	905	50.1	47.4	4889	1483	1465	50.5	47.4	0.4	597	77	44.6	39	54.1
1370	2390	27366	27384	52.2	52.6	4890	27579	27558	51.1	40.9	1.1	214	74.9	44.4	39	52.9
1371	2391	16549	16567	54.9	52.6	4891	16774	16751	53.6	41.7	1.3	226	74	41.6	39	53
1372	2392	19794	19813	50	50	4892	19916	19895	50.2	40.9	0.2	123	72.1	43.1	39	50.6
1373	2393	16551	16568	51.1	50	4893	17062	17045	50.2	50	0.9	512	76	42.4	39	53.3
1374	2394	12726	12746	51.3	47.6	4894	13155	13137	52.1	52.6	0.8	430	76.4	44	39	53.9
1375	2395	545	564	50.7	50	4895	1171	1153	50.4	47.4	0.3	627	78.2	47.2	39	54.9
1376	2396	887	905	50.1	47.4	4896	1483	1464	51.3	45	1.2	597	77	44.6	39	54.1
1377	2397	9927	9945	50.8	52.6	4897	10356	10336	52.4	47.6	1.6	430	75.6	42.1	39	53.3
1378	2398	887	905	50.1	47.4	4898	1481	1463	50.5	47.4	0.4	595	77	44.5	39	54.1
1379	2399	12726	12746	51.3	47.6	4899	12911	12891	51.2	47.6	0.1	186	73.5	41.9	39	51.9
1380	2400	19795	19814	50.4	45	4900	19917	19896	50.9	45.5	0.5	123	72.1	43.1	39	50.7
1381	2401	27361	27380	52.4	55	4901	27566	27546	50.7	47.6	1.7	206	75.1	45.1	39	52.9
1382	2402	27361	27380	52.4	55	4902	27569	27548	50.9	40.9	1.5	209	74.9	44.5	39	52.8
1383	2403	27361	27380	52.4	55	4903	27571	27551	51.4	42.9	1	211	75	44.5	39	53
1384	2404	8867	8886	50.7	50	4904	9256	9237	50.8	45	0.1	390	75.1	41.3	39	52.9
1385	2405	8373	8391	50.7	47.4	4905	9109	9087	50.5	43.5	0.1	737	75.4	40	39	53
1386	2406	19800	19817	50.4	50	4906	19927	19908	52.1	55	1.7	128	72.6	43.8	39	51
1387	2407	19800	19817	50.4	50	4907	19924	19905	50.1	50	0.3	125	72.2	43.2	39	50.7
1388	2408	16553	16571	53.4	52.6	4908	16774	16751	53.6	41.7	0.3	222	73.7	41	39	52.7
1389	2409	2427	2445	52.1	52.6	4909	3053	3034	50.3	50	1.8	627	76.4	42.7	39	53.6
1390	2410	887	905	50.1	47.4	4910	1479	1460	51.6	50	1.5	593	77.1	44.7	39	54.1
1391	2411	13177	13197	50.3	42.9	4911	13321	13301	50.3	42.9	0	145	73.1	43.4	39	51.3
1392	2412	8374	8395	52.4	45.5	4912	9109	9087	50.5	43.5	1.9	736	75.4	40.1	39	53.1
1393	2413	9926	9944	50.5	52.6	4913	10183	10165	51.7	47.4	1.2	258	75.2	43.8	39	52.9

1394	2414	16562	16580	51.9	52.6	4914	17056	17035	51.8	45.5	0.1	495	75.8	42	39	53.7
1395	2415	16562	16581	52.6	50	4915	17056	17035	51.8	45.5	0.8	495	75.8	42	39	53.7
1396	2416	9926	9944	50.5	52.6	4916	10183	10166	50.9	50	0.4	258	75.2	43.8	39	52.9
1397	2417	13177	13197	50.3	42.9	4917	13325	13305	50.5	47.6	0.2	149	73.3	43.6	39	51.5
1398	2418	10141	10160	51	45	4918	10356	10336	52.4	47.6	1.4	216	73.5	40.7	39	51.8
1399	2419	2823	2844	50.4	45.5	4919	3185	3164	51	45.5	0.5	363	75.6	42.7	39	53.1
1400	2420	19800	19818	52.1	52.6	4920	19916	19895	50.2	40.9	1.9	117	71.7	42.7	39	50.3
1401	2421	8063	8084	51.4	45.5	4921	8189	8170	50.6	50	0.8	127	72.3	43.3	39	50.9
1402	2422	985	1008	56.1	50	4922	1485	1465	56	52.4	0	501	76.5	43.7	39	55.4
1403	2423	985	1008	56.1	50	4923	1485	1466	55.6	55	0.5	501	76.5	43.7	39	55.3
1404	2424	985	1008	56.1	50	4924	1495	1474	55.1	45.5	1	511	76.5	43.6	39	55.2
1405	2425	18017	18036	54.8	55	4925	18231	18209	53.5	47.8	1.3	215	74.5	43.3	39	53.3
1406	2426	985	1008	56.1	50	4926	1497	1476	56.4	50	0.3	513	76.6	43.9	39	55.5
1407	2427	13039	13057	51.1	52.6	4927	13155	13137	52.1	52.6	1	117	73.4	47	39	51.8
1408	2428	985	1008	56.1	50	4928	1498	1478	54.9	47.6	1.2	514	76.5	43.8	39	55.1
1409	2429	988	1006	52.2	52.6	4929	1496	1478	50.4	47.4	1.9	509	76.5	43.8	39	53.8
1410	2430	988	1006	52.2	52.6	4930	1497	1480	50.3	50	2	510	76.6	43.9	39	53.8
1411	2431	19856	19875	50.2	45	4931	20033	20016	50.4	50	0.2	178	74.1	43.8	39	52
1412	2432	988	1006	52.2	52.6	4932	1498	1481	51	50	1.2	511	76.6	43.8	39	54
1413	2433	3361	3382	51.9	45.5	4933	3650	3631	53.1	50	1.2	290	75.7	44.1	39	53.6
1414	2434	8867	8888	52.7	45.5	4934	9365	9347	53	52.6	0.3	499	75.8	42.1	39	54
1415	2435	24921	24938	50.4	50	4935	25645	25626	50.8	45	0.4	725	75.5	40.4	39	53.1
1416	2436	3361	3382	51.9	45.5	4936	3647	3628	50.6	45	1.3	287	75.6	43.9	39	53.2
1417	2437	24635	24653	50.5	52.6	4937	25398	25378	51.1	42.9	0.6	764	75.5	40.3	39	53.1
1418	2438	8867	8888	52.7	45.5	4938	9256	9237	50.8	45	1.9	390	75.1	41.3	39	52.9
1419	2439	18017	18036	54.8	55	4939	18712	18693	54.8	55	0	696	76.4	42.5	39	55
1420	2440	24633	24651	50.1	52.6	4940	25398	25378	51.1	42.9	0.9	766	75.6	40.3	39	53
1421	2441	18011	18032	55.7	54.5	4941	18220	18202	54.8	52.6	0.9	210	74.5	43.3	39	53.7
1422	2442	18014	18032	51	52.6	4942	18223	18206	51.8	50	0.8	210	74.3	42.9	39	52.4
1423	2443	24630	24648	50.8	52.6	4943	25398	25378	51.1	42.9	0.2	769	75.6	40.4	39	53.3
1424	2444	18014	18032	51	52.6	4944	18231	18210	52.2	45.5	1.2	218	74.5	43.1	39	52.5
1425	2445	18014	18032	51	52.6	4945	18233	18214	52	50	1.1	220	74.7	43.6	39	52.7
1426	2446	18014	18032	51	52.6	4946	18233	18215	51.3	52.6	0.4	220	74.7	43.6	39	52.7
1427	2447	18011	18031	54.5	52.4	4947	18220	18201	56.1	55	1.6	210	74.5	43.3	39	53.6
1428	2448	3361	3382	51.9	45.5	4948	3646	3625	52	40.9	0.1	286	75.5	43.7	39	53.5
1429	2449	4658	4677	50.5	50	4949	5306	5288	52.4	52.6	2	649	75.5	40.7	39	53.1
1430	2450	18012	18031	53.2	55	4950	18223	18205	53.3	52.6	0.2	212	74.5	43.4	39	53.2
1431	2451	18012	18031	53.2	55	4951	18712	18693	54.8	55	1.7	701	76.4	42.7	39	54.6
1432	2452	13040	13059	50.9	50	4952	13325	13305	50.5	47.6	0.4	286	75.7	44.4	39	53.3
1433	2453	8867	8888	52.7	45.5	4953	9249	9231	50.8	47.4	1.9	383	75.2	41.5	39	53
1434	2454	24179	24198	51	45	4954	24740	24717	52.5	41.7	1.4	562	76	42.2	39	53.6
1435	2455	18013	18031	50.6	52.6	4955	18229	18209	50.1	42.9	0.5	217	74.4	42.9	39	52.2
1436	2456	8865	8884	50.4	45	4956	9340	9319	50.8	45.5	0.3	476	75.5	41.6	39	53.1
1437	2457	24558	24577	50.7	50	4957	24936	24919	51.8	50	1.1	379	75.8	43	39	53.3
1438	2458	8867	8888	52.7	45.5	4958	9249	9230	51.5	45	1.2	383	75.2	41.5	39	53.2
1439	2459	26039	26058	54	55	4959	26753	26733	54	52.4	0.1	715	76	41.5	39	54.5
1440	2460	26039	26058	54	55	4960	26753	26734	52.6	55	1.4	715	76	41.5	39	54.1
1441	2461	18009	18028	51.6	55	4961	18223	18206	51.8	50	0.1	215	74.5	43.3	39	52.7
1442	2462	24482	24503	51.6	40.9	4962	25080	25062	53.5	52.6	1.8	599	75.5	40.7	39	53.4
1443	2463	8861	8880	50.2	45	4963	9109	9087	50.5	43.5	0.4	249	73.8	40.6	39	51.8
1444	2464	24483	24503	51	42.9	4964	25086	25069	50.3	50	0.6	604	75.5	40.7	39	53

1445	2465	18011	18030	52.9	55	4965	18220	18202	54.8	52.6	2	210	74.5	43.3	39	53.1
1446	2466	24481	24502	51.5	45.5	4966	24815	24792	53.4	41.7	1.9	335	75.5	43	39	53.4
1447	2467	24481	24502	51.5	45.5	4967	25081	25063	52.4	52.6	0.9	601	75.5	40.8	39	53.4
1448	2468	24482	24502	50.3	42.9	4968	25082	25064	51.1	52.6	0.8	601	75.5	40.8	39	53
1449	2469	24482	24502	50.3	42.9	4969	25085	25068	50.3	50	0	604	75.4	40.6	39	53
1450	2470	24482	24502	50.3	42.9	4970	25086	25069	50.3	50	0	605	75.5	40.7	39	53
1451	2471	18011	18030	52.9	55	4971	18223	18206	51.8	50	1.1	213	74.4	43.2	39	52.7
1452	2472	18011	18030	52.9	55	4972	18231	18210	52.2	45.5	0.7	221	74.7	43.4	39	53
1453	2473	18011	18030	52.9	55	4973	18233	18214	52	50	0.9	223	74.9	43.9	39	53.1
1454	2474	18011	18030	52.9	55	4974	18233	18215	51.3	52.6	1.6	223	74.9	43.9	39	52.9
1455	2475	24419	24440	52.3	45.5	4975	24815	24792	53.4	41.7	1.2	397	75.9	43.1	39	53.9
1456	2476	18008	18029	54.5	50	4976	18220	18201	56.1	55	1.6	213	74.4	43.2	39	53.6
1457	2477	24420	24440	50.8	42.9	4977	24527	24507	51	42.9	0.2	108	70.7	41.7	39	49.9
1458	2478	12232	12250	51.9	52.6	4978	12994	12976	50.3	47.4	1.6	763	76.4	42.5	39	53.7
1459	2479	4644	4665	52.5	45.5	4979	5306	5288	52.4	52.6	0.1	663	75.6	40.9	39	53.8
1460	2480	18009	18029	53.3	52.4	4980	18712	18693	54.8	55	1.6	704	76.4	42.6	39	54.6
1461	2481	18010	18029	51.8	50	4981	18223	18205	53.3	52.6	1.5	214	74.4	43	39	52.7
1462	2482	24418	24439	52.9	45.5	4982	24527	24507	51	42.9	1.9	110	71.3	42.7	39	50.3
1463	2483	24418	24439	52.9	45.5	4983	24815	24792	53.4	41.7	0.5	398	75.9	43.2	39	54.1
1464	2484	9351	9370	51.2	50	4984	10017	9999	52.8	52.6	1.6	667	75.7	40.9	39	53.4
1465	2485	18011	18029	51.3	52.6	4985	18229	18209	50.1	42.9	1.2	219	74.4	42.9	39	52.2
1466	2486	13176	13196	51.4	47.6	4986	13314	13297	51	50	0.4	139	73	43.9	39	51.5
1467	2487	3229	3248	50.6	50	4987	3497	3478	51.3	50	0.6	269	74.5	41.6	39	52.4
1468	2488	25772	25793	52.4	40.9	4988	26182	26161	51.2	40.9	1.2	411	74.7	40.1	39	52.8
1469	2489	3229	3248	50.6	50	4989	3500	3481	51.2	50	0.5	272	74.5	41.5	39	52.4
1470	2490	13176	13196	51.4	47.6	4990	13323	13304	51.1	45	0.3	148	73.3	43.9	39	51.8
1471	2491	25771	25790	51.1	45	4991	26183	26163	51.7	42.9	0.6	413	74.8	40.4	39	52.8
1472	2492	24418	24436	50	47.4	4992	24526	24506	50.3	42.9	0.3	109	71.4	43.1	39	50.1
1473	2493	25769	25786	50.3	50	4993	26182	26161	51.2	40.9	0.9	414	74.8	40.3	39	52.6
1474	2494	18009	18028	51.6	55	4994	18231	18210	52.2	45.5	0.6	223	74.7	43.5	39	52.9
1475	2495	18009	18028	51.6	55	4995	18233	18214	52	50	0.4	225	74.9	44	39	53
1476	2496	24418	24436	50	47.4	4996	25082	25064	51.1	52.6	1.1	665	75.8	41.2	39	53.2
1477	2497	18009	18028	51.6	55	4997	18233	18215	51.3	52.6	0.3	225	74.9	44	39	53
1478	2498	24418	24436	50	47.4	4998	25209	25190	50.6	50	0.6	792	76.2	41.9	39	53.5
1479	2499	25363	25381	51.1	52.6	4999	25650	25631	51.3	45	0.1	288	74.2	40.6	39	52.4
1480	2500	25363	25381	51.1	52.6	5000	25651	25634	50.4	50	0.7	289	74.3	40.8	39	52.2
1481	2501	25354	25372	50.9	52.6	5001	25548	25531	51.1	50	0.2	195	74.1	43.1	39	52.2
1482	2502	18005	18024	51.1	50	5002	18223	18206	51.8	50	0.6	219	74.4	42.9	39	52.5
1483	2503	18005	18024	51.1	50	5003	18231	18210	52.2	45.5	1.1	227	74.6	43.2	39	52.7
1484	2504	25354	25372	50.9	52.6	5004	25651	25632	52.7	50	1.8	298	74.6	41.3	39	52.6
1485	2505	18005	18024	51.1	50	5005	18233	18215	51.3	52.6	0.2	229	74.9	43.7	39	52.8
1486	2506	18003	18023	53.5	52.4	5006	18712	18693	54.8	55	1.3	710	76.4	42.7	39	54.7
1487	2507	13176	13196	51.4	47.6	5007	13326	13306	50.7	42.9	0.7	151	73.4	43.7	39	51.7
1488	2508	8868	8889	50.4	40.9	5008	9311	9292	50.7	50	0.3	444	75.4	41.4	39	53
1489	2509	25354	25372	50.9	52.6	5009	25832	25811	52.1	50	1.2	479	75	40.3	39	52.9
1490	2510	8375	8396	51.8	45.5	5010	9109	9087	50.5	43.5	1.2	735	75.4	40	39	53
1491	2511	9918	9938	51.4	47.6	5011	10017	9999	52.8	52.6	1.3	100	72.4	47	39	51.2
1492	2512	8375	8396	51.8	45.5	5012	8933	8916	52.2	50	0.4	559	75.1	40.1	39	53.2
1493	2513	17840	17859	50.8	45	5013	18632	18611	50.2	40.9	0.6	793	76.1	41.5	39	53.4
1494	2514	13040	13059	50.9	50	5014	13155	13138	50.4	50	0.5	116	73.2	46.6	39	51.4
1495	2515	25348	25366	51.2	47.4	5015	25650	25631	51.3	45	0.1	303	74.6	41.3	39	52.7

1496	2516	25348	25366	51.2	47.4	5016	25651	25634	50.4	50	0.7	304	74.7	41.4	39	52.5
1497	2517	13040	13059	50.9	50	5017	13178	13157	50.4	40.9	0.5	139	73.6	45.3	39	51.7
1498	2518	17792	17813	51.6	40.9	5018	18223	18205	53.3	52.6	1.7	432	74.9	40.3	39	53
1499	2519	25348	25366	51.2	47.4	5019	25832	25811	52.1	50	0.9	485	75.1	40.4	39	53
1500	2520	25348	25366	51.2	47.4	5020	25833	25812	51.4	45.5	0.2	486	75	40.3	39	53
1501	2521	25347	25365	52	52.6	5021	25651	25632	52.7	50	0.7	305	74.8	41.6	39	53
1502	2522	8868	8889	50.4	40.9	5022	9252	9234	51.4	52.6	1	385	75.1	41.3	39	52.8
1503	2523	17793	17813	50	42.9	5023	18229	18209	50.1	42.9	0.1	437	74.9	40.3	39	52.5
1504	2524	17793	17813	50	42.9	5024	18231	18211	50.6	47.6	0.6	439	75	40.5	39	52.6
1505	2525	17793	17813	50	42.9	5025	18234	18216	51	52.6	1	442	75.1	40.7	39	52.7
1506	2526	17793	17813	50	42.9	5026	18238	18219	50.3	45	0.2	446	75.1	40.8	39	52.7
1507	2527	17793	17813	50	42.9	5027	18239	18220	50	45	0	447	75.1	40.7	39	52.7
1508	2528	24180	24199	50.3	40	5028	24938	24921	50.4	50	0.1	759	75.8	40.8	39	53.2
1509	2529	25348	25365	50.4	50	5029	25832	25811	52.1	50	1.7	485	75.1	40.4	39	52.8
1510	2530	25068	25085	50.3	50	5030	25182	25164	51.4	47.4	1.1	115	73.3	47	39	51.5
1511	2531	29260	29278	51.3	47.4	5031	29414	29395	50.5	50	0.8	155	74.3	45.8	39	52.3
1512	2532	24179	24198	51	45	5032	24933	24913	51.1	42.9	0.1	755	75.8	40.9	39	53.5
1513	2533	17790	17811	51.6	40.9	5033	18223	18205	53.3	52.6	1.7	434	74.9	40.3	39	53
1514	2534	8063	8084	51.4	45.5	5034	8190	8172	50.3	47.4	1.1	128	72.2	43	39	50.8
1515	2535	24178	24197	50.3	40	5035	24936	24919	51.8	50	1.5	759	75.8	41	39	53.2
1516	2536	17791	17811	50	42.9	5036	18229	18209	50.1	42.9	0.1	439	74.9	40.3	39	52.5
1517	2537	17791	17811	50	42.9	5037	18231	18211	50.6	47.6	0.6	441	75	40.6	39	52.6
1518	2538	24174	24194	50.9	42.9	5038	24740	24717	52.5	41.7	1.5	567	76	42.2	39	53.6
1519	2539	24174	24194	50.9	42.9	5039	24933	24913	51.1	42.9	0.2	760	75.8	40.9	39	53.4
1520	2540	17791	17811	50	42.9	5040	18234	18216	51	52.6	1	444	75.1	40.8	39	52.7
1521	2541	17791	17811	50	42.9	5041	18238	18219	50.3	45	0.2	448	75.1	40.8	39	52.7
1522	2542	17791	17811	50	42.9	5042	18239	18220	50	45	0	449	75.1	40.8	39	52.7
1523	2543	24035	24053	52.2	52.6	5043	24526	24506	50.3	42.9	1.9	492	75.4	41.3	39	53
1524	2544	24035	24053	52.2	52.6	5044	24527	24507	51	42.9	1.2	493	75.4	41.2	39	53.2
1525	2545	17607	17628	52.3	40.9	5045	18231	18209	53.5	47.8	1.2	625	75.2	40	39	53.4
1526	2546	29196	29216	52.5	47.6	5046	29358	29339	52.8	50	0.3	163	74.9	46.6	39	53.3
1527	2547	17608	17628	50.9	42.9	5047	18231	18211	50.6	47.6	0.4	624	75.2	40.1	39	52.9
1528	2548	8868	8889	50.4	40.9	5048	9248	9229	50.1	45	0.3	381	75	41.2	39	52.7
1529	2549	17608	17628	50.9	42.9	5049	18234	18216	51	52.6	0	627	75.3	40.2	39	53.1
1530	2550	29196	29215	51.8	50	5050	29358	29339	52.8	50	1	163	74.9	46.6	39	53.1
1531	2551	24023	24044	51.4	40.9	5051	24527	24508	50.5	45	0.9	505	75.4	41	39	53
1532	2552	9409	9428	51.6	45	5052	9989	9968	51	40.9	0.6	581	75.3	40.4	39	53.1
1533	2553	29196	29214	51.1	52.6	5053	29358	29339	52.8	50	1.7	163	74.9	46.6	39	52.8
1534	2554	8861	8880	50.2	45	5054	9257	9238	50.5	45	0.3	397	74.9	40.8	39	52.6
1535	2555	17607	17627	51.6	42.9	5055	18231	18209	53.5	47.8	1.9	625	75.2	40	39	53.2
1536	2556	29195	29213	51.9	52.6	5056	29358	29339	52.8	50	0.9	164	74.8	46.3	39	53
1537	2557	17608	17627	50.2	45	5057	18231	18211	50.6	47.6	0.4	624	75.2	40.1	39	52.8
1538	2558	985	1004	51.1	50	5058	1622	1602	51.6	47.6	0.5	638	77.2	44.7	39	54.4
1539	2559	17608	17627	50.2	45	5059	18234	18216	51	52.6	0.8	627	75.3	40.2	39	52.9
1540	2560	23841	23860	52.1	55	5060	24496	24478	50.7	52.6	1.4	656	76.1	42.1	39	53.6
1541	2561	23841	23860	52.1	55	5061	24498	24479	51.2	50	0.9	658	76.1	42.1	39	53.8
1542	2562	3404	3422	50.5	47.4	5062	3647	3628	50.6	45	0.1	244	74.6	42.6	39	52.5
1543	2563	9349	9367	51.7	52.6	5063	10017	9999	52.8	52.6	1.1	669	75.7	41	39	53.6
1544	2564	23841	23859	50.5	52.6	5064	24496	24478	50.7	52.6	0.2	656	76.1	42.1	39	53.5
1545	2565	25068	25085	50.3	50	5065	25548	25531	51.1	50	0.8	481	75.8	42.2	39	53.3
1546	2566	29186	29205	50.1	40	5066	29414	29395	50.5	50	0.4	229	75.4	45	39	52.9

1547	2567	29182	29204	53.2	43.5	5067	29358	29339	52.8	50	0.4	177	74.6	45.2	39	53.2
1548	2568	23841	23859	50.5	52.6	5068	24498	24479	51.2	50	0.7	658	76.1	42.1	39	53.5
1549	2569	3404	3422	50.5	47.4	5069	3646	3625	52	40.9	1.5	243	74.5	42.4	39	52.4
1550	2570	29183	29204	50.4	40.9	5070	29414	29395	50.5	50	0.2	232	75.4	44.8	39	53
1551	2571	23841	23859	50.5	52.6	5071	24527	24508	50.5	45	0	687	76.1	41.9	39	53.5
1552	2572	23838	23857	50.4	50	5072	24093	24075	50.9	52.6	0.5	256	75.8	45.3	39	53.3
1553	2573	23838	23857	50.4	50	5073	24496	24478	50.7	52.6	0.3	659	76.1	41.9	39	53.4
1554	2574	23838	23857	50.4	50	5074	24498	24479	51.2	50	0.8	661	76.1	41.9	39	53.5
1555	2575	29181	29201	52.4	47.6	5075	29358	29339	52.8	50	0.4	178	74.8	45.5	39	53.2
1556	2576	29181	29201	52.4	47.6	5076	29414	29395	50.5	50	1.9	234	75.6	45.3	39	53.2
1557	2577	29180	29200	51.7	42.9	5077	29358	29339	52.8	50	1.2	179	74.7	45.3	39	52.9
1558	2578	985	1004	51.1	50	5078	1498	1481	51	50	0.1	514	76.5	43.8	39	54
1559	2579	985	1004	51.1	50	5079	1497	1480	50.3	50	0.8	513	76.6	43.9	39	53.8
1560	2580	8859	8879	50	42.9	5080	9254	9236	50.6	47.4	0.6	396	75	40.9	39	52.6
1561	2581	29178	29198	51.4	42.9	5081	29414	29395	50.5	50	0.9	237	75.6	45.1	39	53.2
1562	2582	8859	8879	50	42.9	5082	9340	9319	50.8	45.5	0.7	482	75.5	41.5	39	53
1563	2583	16909	16928	50.8	45	5083	17109	17089	50.4	42.9	0.4	201	74.9	44.8	39	52.7
1564	2584	8794	8813	51.6	45	5084	8919	8901	50.4	47.4	1.2	126	71.8	42.1	39	50.5
1565	2585	8794	8813	51.6	45	5085	8920	8902	52.8	52.6	1.2	127	72	42.5	39	51
1566	2586	985	1004	51.1	50	5086	1496	1478	50.4	47.4	0.7	512	76.5	43.8	39	53.8
1567	2587	18017	18036	54.8	55	5087	18223	18205	53.3	52.6	1.5	207	74.3	43	39	53.1
1568	2588	4593	4613	51.5	47.6	5088	4994	4974	51.2	47.6	0.3	402	76.1	43.5	39	53.7
1569	2589	18017	18036	54.8	55	5089	18220	18201	56.1	55	1.3	204	74.3	43.1	39	53.5
1570	2590	6155	6174	52.1	50	5090	6486	6467	50.8	45	1.2	332	74.5	40.7	39	52.5
1571	2591	6158	6178	51.3	42.9	5091	6486	6467	50.8	45	0.4	329	74.3	40.1	39	52.4
1572	2592	3232	3251	50.3	50	5092	3500	3481	51.2	50	0.8	269	74.5	41.6	39	52.3
1573	2593	3232	3251	50.3	50	5093	3497	3478	51.3	50	1	266	74.5	41.7	39	52.3
1574	2594	28523	28544	51.6	40.9	5094	29298	29279	52.6	55	1	776	78.4	47.3	39	55.5
1575	2595	28965	28984	52.9	55	5095	29358	29339	52.8	50	0.1	394	76.6	44.9	39	54.6
1576	2596	8866	8885	51.1	45	5096	9256	9237	50.8	45	0.3	391	75.1	41.2	39	52.9
1577	2597	28518	28538	51.2	42.9	5097	28672	28654	50.6	52.6	0.7	155	76.7	51.6	39	54
1578	2598	6165	6183	51.2	52.6	5098	6486	6467	50.8	45	0.3	322	74.5	40.7	39	52.5
1579	2599	6264	6283	50.4	50	5099	6483	6463	50.2	42.9	0.2	220	73.8	41.4	39	51.8
1580	2600	18074	18093	50.3	45	5100	18233	18215	51.3	52.6	1	160	73.9	44.4	39	51.9
1581	2601	6271	6291	51.1	47.6	5101	6483	6463	50.2	42.9	0.9	213	73.5	40.8	39	51.6
1582	2602	18074	18093	50.3	45	5102	18231	18210	52.2	45.5	1.9	158	73.5	43.7	39	51.7
1583	2603	6274	6293	50.1	45	5103	6483	6463	50.2	42.9	0.1	210	73.5	41	39	51.6
1584	2604	18074	18093	50.3	45	5104	18223	18206	51.8	50	1.5	150	73.2	43.3	39	51.4
1585	2605	5	23	51.3	52.6	5105	314	296	50.6	47.4	0.6	310	76.8	46.5	39	54
1586	2606	6343	6364	50.7	45.5	5106	6486	6467	50.8	45	0.1	144	71.7	40.3	39	50.5
1587	2607	3800	3820	50.6	42.9	5107	4445	4425	50.6	42.9	0	646	75.4	40.2	39	53
1588	2608	7615	7635	51.1	47.6	5108	7821	7798	52.8	41.7	1.6	207	73.7	41.5	39	52
1589	2609	7723	7741	52.2	52.6	5109	8049	8032	50.4	50	1.8	327	74.9	41.6	39	52.6
1590	2610	1	19	50.1	52.6	5110	314	296	50.6	47.4	0.6	314	76.8	46.5	39	53.9
1591	2611	7725	7742	50	50	5111	7856	7836	51.1	42.9	1.1	132	71.3	40.2	39	50
1592	2612	18074	18094	51.1	42.9	5112	18233	18215	51.3	52.6	0.3	160	73.9	44.4	39	52.1
1593	2613	3168	3189	51	45.5	5113	3503	3484	51.5	50	0.5	336	75.3	42.6	39	53.1
1594	2614	18074	18094	51.1	42.9	5114	18231	18210	52.2	45.5	1.1	158	73.5	43.7	39	51.9
1595	2615	13177	13197	50.3	42.9	5115	13312	13294	51	52.6	0.7	136	72.7	43.4	39	51.1
1596	2616	28190	28209	54.2	55	5116	28671	28652	52.8	55	1.5	482	79.9	52.1	39	56.8
1597	2617	28190	28209	54.2	55	5117	28673	28654	53.5	55	0.7	484	79.9	52.3	39	57.1

1598	2618	28190	28208	51.7	52.6	5118	28671	28652	52.8	55	1	482	79.9	52.1	39	56.5
1599	2619	28190	28208	51.7	52.6	5119	28671	28653	50.2	52.6	1.5	482	79.9	52.1	39	56.1
1600	2620	18074	18094	51.1	42.9	5120	18223	18206	51.8	50	0.7	150	73.2	43.3	39	51.6
1601	2621	28185	28205	53.5	47.6	5121	28284	28265	52.9	50	0.6	100	74.5	52	39	53.1
1602	2622	28187	28205	53.1	52.6	5122	28672	28653	51.8	55	1.2	486	79.9	52.3	39	56.6
1603	2623	2371	2389	50.3	47.4	5123	2900	2881	50.1	45	0.2	530	76.8	44.3	39	53.9
1604	2624	2371	2389	50.3	47.4	5124	3052	3033	50.3	50	0.1	682	76.7	43.4	39	53.9
1605	2625	2371	2389	50.3	47.4	5125	3056	3038	50.8	52.6	0.5	686	76.7	43.4	39	53.9
1606	2626	18074	18095	52.2	45.5	5126	18223	18205	53.3	52.6	1.1	150	73.2	43.3	39	52
1607	2627	2220	2239	51.3	45	5127	2891	2873	50.8	47.4	0.5	672	76.8	43.8	39	54.1
1608	2628	18077	18097	51.5	47.6	5128	18662	18641	50.4	40.9	1.1	586	76.2	42.7	39	53.6
1609	2629	28117	28135	50.6	52.6	5129	28671	28653	50.2	52.6	0.4	555	80	51.9	39	56.1
1610	2630	28116	28134	50.8	47.4	5130	28671	28653	50.2	52.6	0.6	556	79.9	51.8	39	56.1
1611	2631	12232	12250	51.9	52.6	5131	13000	12981	51.1	45	0.8	769	76.5	42.5	39	54
1612	2632	18080	18098	51.2	52.6	5132	18702	18685	50.2	50	1	623	76.2	42.4	39	53.5
1613	2633	12232	12250	51.9	52.6	5133	12999	12980	50.6	40	1.4	768	76.4	42.4	39	53.8
1614	2634	28820	28840	54.8	47.6	5134	29306	29285	56.7	54.5	1.9	487	77.1	45.4	39	55.5
1615	2635	28820	28840	54.8	47.6	5135	29306	29287	54.6	55	0.3	487	77.1	45.4	39	55.5
1616	2636	18080	18098	51.2	52.6	5136	18642	18622	50.5	42.9	0.7	563	76.2	42.6	39	53.6
1617	2637	8865	8884	50.4	45	5137	9249	9231	50.8	47.4	0.4	385	75.1	41.3	39	52.8
1618	2638	8865	8884	50.4	45	5138	9249	9230	51.5	45	1.1	385	75.1	41.3	39	52.8
1619	2639	8865	8884	50.4	45	5139	9109	9087	50.5	43.5	0.1	245	73.9	40.8	39	51.9
1620	2640	28819	28839	56.6	52.4	5140	29306	29285	56.7	54.5	0.2	488	77.2	45.5	39	56.1
1621	2641	9130	9151	52	40.9	5141	9364	9346	53.9	52.6	2	235	74.8	43.4	39	53.1
1622	2642	28820	28839	54.3	50	5142	29306	29287	54.6	55	0.3	487	77.1	45.4	39	55.4
1623	2643	8865	8884	50.4	45	5143	9248	9229	50.1	45	0.3	384	75	41.1	39	52.7
1624	2644	18080	18098	51.2	52.6	5144	18229	18209	50.1	42.9	1.1	150	73.2	43.3	39	51.4
1625	2645	15752	15772	50.8	47.6	5145	16175	16155	51.8	47.6	1	424	75.1	41	39	52.9
1626	2646	12232	12250	51.9	52.6	5146	12498	12480	50	47.4	1.9	267	74.7	42.3	39	52.4
1627	2647	18078	18098	51.5	47.6	5147	18223	18205	53.3	52.6	1.8	146	73	43.2	39	51.6
1628	2648	7833	7853	50.7	47.6	5148	8054	8035	50.4	50	0.2	222	74.6	43.2	39	52.4
1629	2649	230	248	51.2	52.6	5149	713	695	50.7	47.4	0.5	484	79.3	50.6	39	55.8
1630	2650	1472	1491	51.2	45	5150	2153	2134	50.4	45	0.8	682	76.5	42.8	39	53.7
1631	2651	18076	18098	54.4	47.8	5151	18220	18201	56.1	55	1.8	145	73.1	43.4	39	52.6
1632	2652	1442	1461	51.6	55	5152	1694	1673	51.7	40.9	0.1	253	75.9	45.5	39	53.7
1633	2653	28618	28636	52.5	52.6	5153	29358	29339	52.8	50	0.3	741	78.2	47	39	55.6
1634	2654	940	959	56.3	55	5154	1701	1677	54.7	40	1.6	762	77.1	44.2	40	55.5
1635	2655	18076	18097	53.1	45.5	5155	18696	18672	53.9	40	0.8	621	76.2	42.4	40	54.4
1636	2656	940	959	56.3	55	5156	1697	1673	54.4	40	1.9	758	77.2	44.3	40	55.5
1637	2657	3016	3036	50.2	42.9	5157	3188	3167	50.2	40.9	0.1	173	74.5	45.1	40	52.3
1638	2658	18077	18097	51.5	47.6	5158	18696	18673	53.4	41.7	1.9	620	76.2	42.4	40	53.9
1639	2659	18077	18097	51.5	47.6	5159	18697	18679	51.9	52.6	0.3	621	76.2	42.5	40	53.9
1640	2660	9352	9371	50.6	45	5160	9989	9968	51	40.9	0.4	638	75.4	40.3	40	53
1641	2661	6042	6062	50.4	47.6	5161	6374	6353	50	40.9	0.3	333	74.6	40.8	40	52.3
1642	2662	942	960	52.1	52.6	5162	1697	1678	50.3	45	1.8	756	77.2	44.3	40	54.2
1643	2663	942	960	52.1	52.6	5163	1697	1677	51	42.9	1.1	756	77.2	44.3	40	54.4
1644	2664	6042	6062	50.4	47.6	5164	6292	6273	50.8	45	0.4	251	73.9	40.6	40	51.9
1645	2665	942	960	52.1	52.6	5165	1697	1676	51.7	40.9	0.5	756	77.2	44.3	40	54.6
1646	2666	942	960	52.1	52.6	5166	1694	1673	51.7	40.9	0.4	753	77.2	44.4	40	54.7
1647	2667	13176	13196	51.4	47.6	5167	13749	13727	50.5	43.5	0.9	574	76.2	42.7	40	53.6
1648	2668	13176	13196	51.4	47.6	5168	13949	13932	51.6	50	0.2	774	75.9	41.1	40	53.6

1649	2669	942	960	52.1	52.6	5169	1493	1473	52	47.6	0.1	552	76.9	44.4	40	54.5
1650	2670	6042	6062	50.4	47.6	5170	6292	6272	51.5	42.9	1.1	251	73.9	40.6	40	51.9
1651	2671	6042	6062	50.4	47.6	5171	6290	6270	50.9	42.9	0.6	249	73.8	40.6	40	51.9
1652	2672	6042	6062	50.4	47.6	5172	6289	6267	52.2	43.5	1.8	248	73.9	40.7	40	51.9
1653	2673	9402	9420	51.3	47.4	5173	10017	9999	52.8	52.6	1.4	616	75.6	41.1	40	53.5
1654	2674	943	961	50.3	47.4	5174	1697	1678	50.3	45	0	755	77.1	44.2	40	54.2
1655	2675	9139	9159	52.5	47.6	5175	9324	9300	52.9	40	0.4	186	73.9	43	40	52.6
1656	2676	9139	9159	52.5	47.6	5176	9324	9301	52.4	41.7	0.1	186	73.9	43	40	52.6
1657	2677	943	961	50.3	47.4	5177	1697	1677	51	42.9	0.7	755	77.1	44.2	40	54.2
1658	2678	6222	6246	52.2	40	5178	6486	6467	50.8	45	1.4	265	73.8	40	40	52
1659	2679	3895	3914	50.3	45	5179	4608	4590	51.5	52.6	1.2	714	75.5	40.3	40	53
1660	2680	3889	3911	54.2	47.8	5180	4610	4590	53.2	52.4	1.1	722	75.5	40.4	40	53.9
1661	2681	3889	3908	51.3	50	5181	4608	4590	51.5	52.6	0.3	720	75.5	40.4	40	53.4
1662	2682	9139	9159	52.5	47.6	5182	9359	9335	54.5	40	1.9	221	74.7	43.4	40	53.1
1663	2683	943	961	50.3	47.4	5183	1697	1676	51.7	40.9	1.4	755	77.1	44.2	40	54.2
1664	2684	943	961	50.3	47.4	5184	1694	1673	51.7	40.9	1.5	752	77.2	44.3	40	54.2
1665	2685	6302	6321	51.4	50	5185	6483	6463	50.2	42.9	1.2	182	72.9	40.7	40	51.2
1666	2686	9409	9428	51.6	45	5186	10017	9999	52.8	52.6	1.2	609	75.6	41.1	40	53.5
1667	2687	943	961	50.3	47.4	5187	1493	1473	52	47.6	1.7	551	76.8	44.3	40	54
1668	2688	13039	13058	51.8	50	5188	13312	13294	51	52.6	0.8	274	75.7	44.5	40	53.4
1669	2689	3799	3820	52.9	45.5	5189	4565	4542	53.9	41.7	1	767	75.5	40.2	40	53.8
1670	2690	985	1004	51.1	50	5190	1481	1463	50.5	47.4	0.6	497	76.4	43.5	40	53.7
1671	2691	13039	13058	51.8	50	5191	13325	13305	50.5	47.6	1.3	287	75.8	44.6	40	53.3
1672	2692	7615	7635	51.1	47.6	5192	8049	8032	50.4	50	0.8	435	75.7	42.3	40	53.2
1673	2693	7615	7635	51.1	47.6	5193	7853	7833	50.7	47.6	0.4	239	74.4	42.3	40	52.4
1674	2694	3034	3053	50.3	50	5194	3503	3484	51.5	50	1.2	470	76.3	43.4	40	53.6
1675	2695	9140	9159	50.1	45	5195	9334	9315	52.1	50	2	195	74.3	43.6	40	52.1
1676	2696	3799	3819	51.3	47.6	5196	4186	4168	51.8	52.6	0.5	388	75.3	41.8	40	53.2
1677	2697	3799	3819	51.3	47.6	5197	4434	4416	51.5	52.6	0.2	636	75.3	40.3	40	53.2
1678	2698	3799	3819	51.3	47.6	5198	4435	4417	50.5	52.6	0.8	637	75.4	40.3	40	53
1679	2699	7617	7636	50.9	50	5199	8190	8172	50.3	47.4	0.6	574	76.1	42.3	40	53.4
1680	2700	18011	18032	55.7	54.5	5200	18443	18424	55.9	55	0.2	433	76.1	43.2	40	55.1
1681	2701	18013	18032	52.2	55	5201	18696	18672	53.9	40	1.7	684	76.3	42.4	40	54.2
1682	2702	18013	18032	52.2	55	5202	18696	18673	53.4	41.7	1.2	684	76.3	42.4	40	54.2
1683	2703	13177	13197	50.3	42.9	5203	13545	13527	50.3	52.6	0	369	77	46.1	40	54.1
1684	2704	9922	9941	51.3	50	5204	10455	10434	51.1	40.9	0.1	534	75.3	40.6	40	53.1
1685	2705	7617	7636	50.9	50	5205	7853	7833	50.7	47.6	0.3	237	74.4	42.2	40	52.4
1686	2706	13177	13197	50.3	42.9	5206	13329	13308	50.5	40.9	0.2	153	73.2	43.1	40	51.4
1687	2707	18014	18032	51	52.6	5207	18238	18219	50.3	45	0.7	225	74.8	43.6	40	52.5
1688	2708	18014	18032	51	52.6	5208	18239	18220	50	45	0.9	226	74.7	43.4	40	52.4
1689	2709	18014	18032	51	52.6	5209	18697	18679	51.9	52.6	0.9	684	76.3	42.4	40	53.8
1690	2710	7708	7730	50.6	43.5	5210	7853	7833	50.7	47.6	0.1	146	71.8	40.4	40	50.6
1691	2711	9140	9159	50.1	45	5211	9358	9338	51	42.9	0.9	219	74.4	42.9	40	52.2
1692	2712	7723	7741	52.2	52.6	5212	7856	7836	51.1	42.9	1.1	134	71.4	40.3	40	50.4
1693	2713	988	1006	52.2	52.6	5213	1171	1153	50.4	47.4	1.8	184	73.8	42.9	40	51.9
1694	2714	13177	13197	50.3	42.9	5214	13328	13307	51.2	45.5	0.9	152	73.3	43.4	40	51.5
1695	2715	9935	9955	50.4	42.9	5215	10608	10589	51	50	0.6	674	75.8	41.1	40	53.2
1696	2716	985	1008	56.1	50	5216	1484	1463	55.5	50	0.5	500	76.4	43.6	40	55.3
1697	2717	13033	13051	52.1	52.6	5217	13179	13158	50.4	40.9	1.7	147	74.3	46.3	40	52.2
1698	2718	12977	12996	50.2	40	5218	13320	13300	51.4	47.6	1.1	344	76.1	44.2	40	53.4
1699	2719	12977	12996	50.2	40	5219	13321	13301	50.3	42.9	0.1	345	76	44.1	40	53.4

1700	2720	2823	2844	50.4	45.5	5220	3192	3171	51.9	50	1.5	370	75.7	43	40	53.2
1701	2721	18009	18030	54.6	54.5	5221	18443	18424	55.9	55	1.4	435	76.1	43.2	40	54.7
1702	2722	12976	12995	51.1	45	5222	13320	13300	51.4	47.6	0.3	345	76.1	44.3	40	53.7
1703	2723	1046	1064	51.2	47.4	5223	1531	1512	52.7	55	1.5	486	76.7	44.2	40	54.1
1704	2724	12976	12995	51.1	45	5224	13321	13301	50.3	42.9	0.8	346	76.1	44.2	40	53.5
1705	2725	12976	12994	50.3	47.4	5225	13320	13300	51.4	47.6	1.1	345	76.1	44.3	40	53.5
1706	2726	12976	12994	50.3	47.4	5226	13321	13301	50.3	42.9	0	346	76.1	44.2	40	53.5
1707	2727	18011	18030	52.9	55	5227	18696	18672	53.9	40	1	686	76.3	42.4	40	54.4
1708	2728	18011	18030	52.9	55	5228	18696	18673	53.4	41.7	0.5	686	76.3	42.4	40	54.4
1709	2729	18011	18030	52.9	55	5229	18697	18679	51.9	52.6	1	687	76.3	42.5	40	54.1
1710	2730	9140	9159	50.1	45	5230	9374	9353	50.1	40.9	0	235	74.5	42.6	40	52.3
1711	2731	3	23	55.4	52.4	5231	204	185	56.6	55	1.3	202	75	45	40	54.2
1712	2732	15255	15273	50.3	52.6	5232	15761	15741	51.7	47.6	1.4	507	75	40	40	52.7
1713	2733	15255	15273	50.3	52.6	5233	15763	15743	52	47.6	1.7	509	75	40.1	40	52.7
1714	2734	12965	12985	51.2	42.9	5234	13320	13300	51.4	47.6	0.2	356	76.1	44.1	40	53.7
1715	2735	8373	8391	50.7	47.4	5235	9060	9039	50.3	40.9	0.4	688	75.4	40.1	40	53
1716	2736	12962	12980	50.7	47.4	5236	13320	13300	51.4	47.6	0.7	359	76.2	44.3	40	53.6
1717	2737	12938	12957	50.9	45	5237	13155	13137	52.1	52.6	1.2	218	75.4	45.4	40	53.2
1718	2738	2671	2692	52.1	40.9	5238	3190	3169	50.7	45.5	1.5	520	75.6	41.5	40	53.2
1719	2739	2671	2692	52.1	40.9	5239	3192	3171	51.9	50	0.2	522	75.7	41.8	40	53.7
1720	2740	12938	12956	50.1	47.4	5240	13155	13137	52.1	52.6	2	218	75.4	45.4	40	52.9
1721	2741	26421	26441	51.5	42.9	5241	26592	26574	52.4	52.6	0.9	172	72.4	40.1	40	51.2
1722	2742	18006	18028	54.5	52.2	5242	18443	18424	55.9	55	1.4	438	76.1	43.2	40	54.7
1723	2743	26421	26441	51.5	42.9	5243	26656	26635	52.9	45.5	1.4	236	74.2	41.9	40	52.5
1724	2744	3055	3074	51.1	50	5244	3210	3190	50.5	47.6	0.6	156	74.2	45.5	40	52.2
1725	2745	7833	7853	50.7	47.6	5245	8189	8170	50.6	50	0	357	75.6	42.9	40	53.2
1726	2746	26421	26441	51.5	42.9	5246	26658	26640	50.8	47.4	0.7	238	74.1	41.6	40	52.2
1727	2747	9131	9151	50.4	42.9	5247	9328	9310	51	52.6	0.7	198	74.3	43.4	40	52.2
1728	2748	24921	24938	50.4	50	5248	25650	25631	51.3	45	0.9	730	75.5	40.4	40	53.1
1729	2749	24921	24938	50.4	50	5249	25651	25634	50.4	50	0	731	75.6	40.5	40	53.1
1730	2750	9130	9151	52	40.9	5250	9324	9301	52.4	41.7	0.5	195	73.9	42.6	40	52.4
1731	2751	9130	9151	52	40.9	5251	9324	9300	52.9	40	1	195	73.9	42.6	40	52.4
1732	2752	8376	8396	50.6	42.9	5252	9107	9086	51.6	45.5	1	732	75.4	40	40	53.1
1733	2753	11541	11561	50.9	42.9	5253	11727	11708	50.4	45	0.5	187	73	40.6	40	51.3
1734	2754	11540	11561	53.8	45.5	5254	11984	11966	53	52.6	0.7	445	75.1	40.7	40	53.6
1735	2755	2371	2389	50.3	47.4	5255	2672	2654	50.9	52.6	0.5	302	77.1	47.4	40	54.2
1736	2756	2371	2389	50.3	47.4	5256	2998	2977	51.1	40.9	0.8	628	76.7	43.5	40	53.9
1737	2757	11543	11562	50.4	40	5257	11727	11708	50.4	45	0.1	185	72.9	40.5	40	51.2
1738	2758	26040	26061	56.4	54.5	5258	26589	26567	56.1	47.8	0.4	550	75.1	40	40	54.5
1739	2759	11541	11562	51.5	40.9	5259	11984	11966	53	52.6	1.5	444	75	40.5	40	53.1
1740	2760	7728	7746	51.7	52.6	5260	8187	8167	50.4	42.9	1.3	460	75.6	42	40	53.2
1741	2761	26040	26061	56.4	54.5	5261	26657	26634	54.6	41.7	1.9	618	75.5	40.6	40	54.3
1742	2762	2223	2243	50.2	42.9	5262	2675	2656	50.4	50	0.2	453	77	45.3	40	54
1743	2763	2220	2239	51.3	45	5263	2676	2657	50.7	50	0.5	457	76.9	45.1	40	54.1
1744	2764	11541	11560	50.1	45	5264	11727	11707	51.1	42.9	1	187	73	40.6	40	51.2
1745	2765	24559	24580	54.2	54.5	5265	25088	25070	54.5	52.6	0.2	530	75.5	41.1	40	54.2
1746	2766	12233	12251	51.1	52.6	5266	12998	12979	50.1	45	1	766	76.5	42.6	40	53.7
1747	2767	12233	12251	51.1	52.6	5267	12412	12392	50	42.9	1.1	180	73.2	41.7	40	51.4
1748	2768	24562	24580	50.1	52.6	5268	25086	25069	50.3	50	0.2	525	75.4	41	40	52.9
1749	2769	9931	9950	50.2	45	5269	10608	10589	51	50	0.8	678	75.8	41.2	40	53.2
1750	2770	24562	24580	50.1	52.6	5270	25209	25188	52	45.5	1.9	648	76.1	42	40	53.4

1751	2771	24562	24580	50.1	52.6	5271	25209	25189	51.4	47.6	1.3	648	76.1	42	40	53.4
1752	2772	3789	3807	51.8	52.6	5272	4445	4425	50.6	42.9	1.2	657	75.5	40.5	40	53.1
1753	2773	26039	26058	54	55	5273	26656	26634	53.4	43.5	0.6	618	75.5	40.6	40	53.9
1754	2774	26039	26058	54	55	5274	26660	26639	52.5	45.5	1.5	622	75.4	40.5	40	53.7
1755	2775	3789	3807	51.8	52.6	5275	4444	4424	50.6	42.9	1.2	656	75.5	40.5	40	53.1
1756	2776	24559	24579	52	52.4	5276	25086	25069	50.3	50	1.6	528	75.5	41.1	40	53
1757	2777	12235	12253	50.1	52.6	5277	12999	12980	50.6	40	0.5	765	76.4	42.5	40	53.6
1758	2778	24559	24579	52	52.4	5278	25209	25188	52	45.5	0	651	76.1	42.1	40	54
1759	2779	24559	24579	52	52.4	5279	25209	25189	51.4	47.6	0.6	651	76.1	42.1	40	53.8
1760	2780	887	905	50.1	47.4	5280	1499	1482	50.1	50	0.1	613	77.1	44.7	40	54.1
1761	2781	24558	24577	50.7	50	5281	25182	25164	51.4	47.4	0.7	625	76	41.9	40	53.5
1762	2782	26039	26058	54	55	5282	26828	26810	52.9	52.6	1.2	790	76.4	42.4	40	54.5
1763	2783	8866	8885	51.1	45	5283	9597	9577	50.3	42.9	0.8	732	75.8	41.1	40	53.3
1764	2784	7727	7745	50.8	47.4	5284	8049	8032	50.4	50	0.5	323	74.8	41.5	40	52.6
1765	2785	13177	13197	50.3	42.9	5285	13747	13726	50.8	40.9	0.4	571	76.2	42.6	40	53.5
1766	2786	887	905	50.1	47.4	5286	1498	1481	51	50	0.9	612	77.2	44.8	40	54.1
1767	2787	1784	1803	52.5	50	5287	2103	2083	50.6	42.9	2	320	76	44.4	40	53.5
1768	2788	12235	12253	50.1	52.6	5288	12498	12480	50	47.4	0.1	264	74.7	42.4	40	52.4
1769	2789	1784	1802	51.8	52.6	5289	2103	2083	50.6	42.9	1.3	320	76	44.4	40	53.5
1770	2790	887	905	50.1	47.4	5290	1496	1478	50.4	47.4	0.3	610	77.1	44.8	40	54.1
1771	2791	1783	1801	52.9	52.6	5291	2153	2133	52.1	42.9	0.9	371	76	43.7	40	53.9
1772	2792	887	905	50.1	47.4	5292	1494	1476	50.7	47.4	0.6	608	77.1	44.7	40	54.1
1773	2793	3791	3809	51.8	52.6	5293	4445	4425	50.6	42.9	1.2	655	75.5	40.5	40	53.1
1774	2794	17813	17832	50.1	45	5294	18506	18488	51.2	52.6	1.2	694	75.8	41.2	40	53.2
1775	2795	3791	3809	51.8	52.6	5295	4444	4424	50.6	42.9	1.2	654	75.5	40.5	40	53.1
1776	2796	17840	17859	50.8	45	5296	18233	18215	51.3	52.6	0.5	394	74.9	40.9	40	52.8
1777	2797	17840	17859	50.8	45	5297	18233	18214	52	50	1.3	394	74.9	40.9	40	52.8
1778	2798	9930	9949	52.2	50	5298	10356	10336	52.4	47.6	0.2	427	75.6	42.2	40	53.7
1779	2799	15211	15230	50.2	45	5299	15949	15930	51.1	45	0.9	739	75.5	40.3	40	53
1780	2800	98	118	50.6	42.9	5300	253	233	51.8	47.6	1.2	156	74.5	46.2	40	52.4
1781	2801	18004	18023	51.1	50	5301	18233	18214	52	50	0.9	230	75	43.9	40	52.9
1782	2802	24420	24440	50.8	42.9	5302	24936	24919	51.8	50	1	517	75.9	42.2	40	53.5
1783	2803	24420	24440	50.8	42.9	5303	24938	24921	50.4	50	0.4	519	75.8	42	40	53.3
1784	2804	98	118	50.6	42.9	5304	254	235	50	45	0.6	157	74.4	45.9	40	52.2
1785	2805	11540	11557	50.4	50	5305	11727	11707	51.1	42.9	0.7	188	73.1	41	40	51.4
1786	2806	98	118	50.6	42.9	5306	642	622	51.6	47.6	0.9	545	79.1	49.7	40	55.6
1787	2807	15211	15230	50.2	45	5307	15595	15576	50.8	45	0.6	385	75.1	41.3	40	52.7
1788	2808	15255	15273	50.3	52.6	5308	15767	15747	50	42.9	0.3	513	75.1	40.2	40	52.6
1789	2809	12373	12391	50.8	47.4	5309	12911	12891	51.2	47.6	0.4	539	76.1	42.5	40	53.6
1790	2810	18009	18028	51.6	55	5310	18697	18679	51.9	52.6	0.2	689	76.4	42.5	40	54
1791	2811	18009	18028	51.6	55	5311	18696	18673	53.4	41.7	1.8	688	76.3	42.4	40	54
1792	2812	18009	18028	51.6	55	5312	18239	18220	50	45	1.6	231	74.9	43.7	40	52.5
1793	2813	18009	18028	51.6	55	5313	18238	18219	50.3	45	1.4	230	75	43.9	40	52.7
1794	2814	24417	24436	52.6	50	5314	25079	25061	52.7	52.6	0.1	663	75.8	41.3	40	54
1795	2815	10250	10271	50.6	45.5	5315	10356	10336	52.4	47.6	1.8	107	70.8	42.1	40	49.9
1796	2816	1356	1375	53.8	55	5316	1484	1464	54.3	47.6	0.5	129	74.4	48.1	40	53.3
1797	2817	1356	1375	53.8	55	5317	1484	1465	53.8	50	0	129	74.4	48.1	40	53.3
1798	2818	1356	1375	53.8	55	5318	1484	1466	53.1	52.6	0.7	129	74.4	48.1	40	53.1
1799	2819	24418	24436	50	47.4	5319	24560	24542	50.2	47.4	0.1	143	72.4	42	40	50.8
1800	2820	18008	18028	53	52.4	5320	18696	18672	53.9	40	0.9	689	76.3	42.4	40	54.4
1801	2821	25363	25381	51.1	52.6	5321	25646	25627	50.5	45	0.7	284	74.1	40.5	40	52.1

1802	2822	9131	9151	50.4	42.9	5322	9333	9315	52.2	52.6	1.9	203	74.6	43.8	40	52.4
1803	2823	9131	9151	50.4	42.9	5323	9374	9353	50.1	40.9	0.3	244	74.6	42.6	40	52.4
1804	2824	24380	24399	55	55	5324	24582	24560	54.2	52.2	0.9	203	74.4	43.3	40	53.4
1805	2825	19802	19820	53	52.6	5325	19921	19900	51.8	45.5	1.2	120	72.4	44.2	40	51.3
1806	2826	3055	3075	51.8	47.6	5326	3210	3190	50.5	47.6	1.3	156	74.2	45.5	40	52.2
1807	2827	3055	3075	51.8	47.6	5327	3207	3187	50.5	47.6	1.3	153	74	45.1	40	52
1808	2828	3055	3076	52.4	45.5	5328	3210	3190	50.5	47.6	2	156	74.2	45.5	40	52.2
1809	2829	24379	24398	55	55	5329	24582	24560	54.2	52.2	0.9	204	74.3	43.1	40	53.3
1810	2830	7876	7895	51.5	45	5330	8054	8035	50.4	50	1.1	179	73.5	42.5	40	51.7
1811	2831	3055	3076	52.4	45.5	5331	3207	3187	50.5	47.6	2	153	74	45.1	40	52
1812	2832	9130	9150	51.3	42.9	5332	9324	9300	52.9	40	1.6	195	73.9	42.6	40	52.2
1813	2833	9130	9150	51.3	42.9	5333	9324	9301	52.4	41.7	1.1	195	73.9	42.6	40	52.2
1814	2834	8794	8813	51.6	45	5334	9324	9301	52.4	41.7	0.8	531	75.7	41.6	40	53.6
1815	2835	8794	8813	51.6	45	5335	9324	9300	52.9	40	1.3	531	75.7	41.6	40	53.6
1816	2836	9130	9150	51.3	42.9	5336	9328	9310	51	52.6	0.3	199	74.2	43.2	40	52.4
1817	2837	9130	9150	51.3	42.9	5337	9333	9315	52.2	52.6	0.9	204	74.5	43.6	40	52.6
1818	2838	24179	24200	53.3	40.9	5338	24807	24786	51.7	45.5	1.6	629	75.8	41.3	40	53.7
1819	2839	4593	4613	51.5	47.6	5339	4708	4690	50.3	47.4	1.3	116	71.4	42.2	40	50.2
1820	2840	9130	9150	51.3	42.9	5340	9374	9353	50.1	40.9	1.2	245	74.6	42.4	40	52.3
1821	2841	29180	29199	50.1	40	5341	29412	29393	50.3	45	0.2	233	75.5	45.1	40	53
1822	2842	25348	25366	51.2	47.4	5342	25772	25753	51.9	50	0.8	425	74.9	40.5	40	52.9
1823	2843	24179	24200	53.3	40.9	5343	24815	24792	53.4	41.7	0.2	637	75.8	41.3	40	54.1
1824	2844	8794	8813	51.6	45	5344	9101	9081	50.5	47.6	1.2	308	74.8	41.6	40	52.6
1825	2845	16861	16880	50.8	50	5345	17056	17035	51.8	45.5	1	196	74.7	44.4	40	52.6
1826	2846	16562	16581	52.6	50	5346	17038	17021	50.7	50	1.9	477	75.7	41.9	40	53.3
1827	2847	16562	16581	52.6	50	5347	17039	17022	51.4	50	1.2	478	75.6	41.8	40	53.5
1828	2848	16562	16581	52.6	50	5348	17041	17023	53.5	52.6	0.9	480	75.7	41.9	40	53.8
1829	2849	3090	3110	50.3	42.9	5349	3647	3628	50.6	45	0.3	558	76.2	42.7	40	53.5
1830	2850	16562	16580	51.9	52.6	5350	17038	17021	50.7	50	1.2	477	75.7	41.9	40	53.3
1831	2851	16562	16580	51.9	52.6	5351	17039	17022	51.4	50	0.5	478	75.6	41.8	40	53.5
1832	2852	24178	24198	52.7	42.9	5352	24815	24792	53.4	41.7	0.7	638	75.7	41.2	40	53.9
1833	2853	16562	16580	51.9	52.6	5353	17041	17023	53.5	52.6	1.6	480	75.7	41.9	40	53.6
1834	2854	24179	24198	51	45	5354	24807	24786	51.7	45.5	0.7	629	75.8	41.3	40	53.5
1835	2855	24179	24198	51	45	5355	24818	24797	51.6	40.9	0.6	640	75.8	41.2	40	53.4
1836	2856	3090	3110	50.3	42.9	5356	3646	3625	52	40.9	1.7	557	76.1	42.5	40	53.5
1837	2857	3089	3110	51.8	45.5	5357	3650	3631	53.1	50	1.3	562	76.3	42.9	40	54
1838	2858	29259	29279	54	52.4	5358	29358	29339	52.8	50	1.1	100	72.4	47	40	51.6
1839	2859	8794	8813	51.6	45	5359	8928	8911	51.9	50	0.2	135	72.2	42.2	40	51.1
1840	2860	24176	24197	52.1	40.9	5360	24815	24792	53.4	41.7	1.3	640	75.8	41.2	40	53.8
1841	2861	29259	29277	50.9	52.6	5361	29358	29339	52.8	50	2	100	72.4	47	40	51.1
1842	2862	29257	29276	51.3	50	5362	29358	29339	52.8	50	1.5	102	72.6	47.1	40	51.3
1843	2863	9915	9935	51.8	47.6	5363	10017	9999	52.8	52.6	1	103	72.9	47.6	40	51.6
1844	2864	4639	4659	51.1	47.6	5364	5306	5288	52.4	52.6	1.3	668	75.6	40.9	40	53.4
1845	2865	24178	24197	50.3	40	5365	24807	24786	51.7	45.5	1.4	630	75.8	41.3	40	53.2
1846	2866	28653	28671	50.2	52.6	5366	29414	29395	50.5	50	0.3	762	78	46.2	40	54.7
1847	2867	28653	28671	50.2	52.6	5367	29412	29393	50.3	45	0.1	760	78	46.2	40	54.7
1848	2868	28652	28671	52.8	55	5368	29358	29339	52.8	50	0	707	78	46.4	40	55.5
1849	2869	15752	15772	50.8	47.6	5369	16213	16195	50.8	52.6	0	462	75.4	41.3	40	53.1
1850	2870	24178	24197	50.3	40	5370	24818	24797	51.6	40.9	1.4	641	75.7	41.2	40	53.2
1851	2871	19794	19814	51.7	47.6	5371	19909	19885	52.5	40	0.8	116	71.8	43.1	40	50.8
1852	2872	8866	8885	51.1	45	5372	9341	9322	51.1	50	0	476	75.6	41.8	40	53.4

1853	2873	15951	15973	52.1	43.5	5373	16175	16155	51.8	47.6	0.3	225	73.7	40.9	40	52.2
1854	2874	24174	24195	52.5	40.9	5374	24815	24791	54.5	40	2	642	75.8	41.3	40	53.9
1855	2875	8866	8885	51.1	45	5375	9340	9319	50.8	45.5	0.3	475	75.6	41.7	40	53.2
1856	2876	15951	15973	52.1	43.5	5376	16169	16151	51.3	52.6	0.8	219	73.5	40.6	40	51.9
1857	2877	15951	15974	53.3	41.7	5377	16175	16154	53.4	45.5	0.1	225	73.7	40.9	40	52.7
1858	2878	27437	27456	50.2	40	5378	27541	27521	51.7	47.6	1.5	105	71.8	44.8	40	50.4
1859	2879	15650	15674	52.9	40	5379	16210	16192	54.3	52.6	1.4	561	75.1	40.1	40	53.6
1860	2880	8866	8885	51.1	45	5380	9334	9316	51.3	52.6	0.2	469	75.7	42	40	53.4
1861	2881	8866	8885	51.1	45	5381	9310	9291	51.2	45	0.1	445	75.3	41.3	40	53.2
1862	2882	8866	8885	51.1	45	5382	9252	9234	51.4	52.6	0.3	387	75.1	41.3	40	53
1863	2883	3360	3379	50.7	45	5383	3494	3473	50.4	40.9	0.3	135	73.7	45.9	40	51.8
1864	2884	8866	8885	51.1	45	5384	9248	9229	50.1	45	1	383	75.1	41.3	40	52.7
1865	2885	18081	18099	51.2	52.6	5385	18697	18679	51.9	52.6	0.7	617	76.3	42.6	40	53.9
1866	2886	8865	8884	50.4	45	5386	9257	9238	50.5	45	0.1	393	75	41	40	52.7
1867	2887	18081	18099	51.2	52.6	5387	18239	18220	50	45	1.2	159	74	44.7	40	51.9
1868	2888	18081	18099	51.2	52.6	5388	18238	18219	50.3	45	0.9	158	74.1	44.9	40	52
1869	2889	28117	28135	50.6	52.6	5389	28505	28487	50.2	47.4	0.4	389	79.5	51.9	40	55.8
1870	2890	8866	8885	51.1	45	5390	9109	9087	50.5	43.5	0.6	244	73.9	41	40	52
1871	2891	9055	9079	52.8	40	5391	9724	9706	51.3	52.6	1.5	670	75.4	40.3	40	53.3
1872	2892	3403	3423	54.1	47.6	5392	3502	3478	55.8	48	1.7	100	71.6	45	40	51.5
1873	2893	28855	28874	52.9	50	5393	29306	29288	53.5	52.6	0.6	452	77.1	45.6	40	54.9
1874	2894	24173	24194	52.5	40.9	5394	24815	24792	53.4	41.7	0.9	643	75.8	41.2	40	53.9
1875	2895	3094	3113	50	50	5395	3647	3628	50.6	45	0.6	554	76.2	42.8	40	53.5
1876	2896	24174	24194	50.9	42.9	5396	24807	24786	51.7	45.5	0.8	634	75.8	41.3	40	53.4
1877	2897	28856	28875	52.2	50	5397	29306	29288	53.5	52.6	1.3	451	77.1	45.7	40	54.7
1878	2898	24174	24194	50.9	42.9	5398	24818	24797	51.6	40.9	0.7	645	75.8	41.2	40	53.4
1879	2899	28857	28876	51.7	45	5399	29306	29288	53.5	52.6	1.8	450	77.1	45.6	40	54.6
1880	2900	8858	8877	51.2	45	5400	9254	9236	50.6	47.4	0.6	397	75	41.1	40	52.8
1881	2901	16553	16571	53.4	52.6	5401	16777	16758	51.5	50	1.9	225	73.7	40.9	40	52.1
1882	2902	29197	29219	54.8	47.8	5402	29301	29282	55.3	55	0.5	105	73.4	48.6	40	52.9
1883	2903	29198	29219	52.6	45.5	5403	29306	29288	53.5	52.6	0.9	109	73.3	47.7	40	52.2
1884	2904	28857	28877	52.3	42.9	5404	29306	29288	53.5	52.6	1.2	450	77.1	45.6	40	54.8
1885	2905	29199	29219	51.2	42.9	5405	29298	29280	51.4	52.6	0.2	100	72.4	47	40	51.1
1886	2906	3094	3113	50	50	5406	3646	3625	52	40.9	2	553	76.2	42.7	40	53.4
1887	2907	3224	3243	52.3	50	5407	3650	3631	53.1	50	0.8	427	75.5	41.9	40	53.7
1888	2908	29195	29216	53.8	45.5	5408	29306	29287	54.6	55	0.8	112	73.6	48.2	40	52.8
1889	2909	28867	28885	51.5	52.6	5409	29358	29339	52.8	50	1.4	492	76.9	44.9	40	54.4
1890	2910	29196	29216	52.5	47.6	5410	29298	29279	52.6	55	0.1	103	73.3	48.5	40	52.1
1891	2911	28867	28886	53.2	50	5411	29415	29395	53.4	52.4	0.2	549	77.1	45	40	55
1892	2912	3093	3113	51.7	47.6	5412	3650	3631	53.1	50	1.4	558	76.3	42.8	40	54
1893	2913	3225	3243	50.9	52.6	5413	3646	3625	52	40.9	1.2	422	75.4	41.7	40	53.1
1894	2914	3225	3243	50.9	52.6	5414	3647	3628	50.6	45	0.3	423	75.5	41.8	40	53.1
1895	2915	28867	28886	53.2	50	5415	29306	29287	54.6	55	1.4	440	76.9	45.2	40	54.9
1896	2916	3223	3241	50.2	52.6	5416	3500	3481	51.2	50	1	278	74.7	42.1	40	52.5
1897	2917	28867	28886	53.2	50	5417	29298	29279	52.6	55	0.5	432	76.8	45.1	40	54.7
1898	2918	24034	24053	53.4	55	5418	24815	24791	54.5	40	1.1	782	76.3	42.1	40	54.5
1899	2919	3221	3239	51.5	52.6	5419	3650	3631	53.1	50	1.6	430	75.5	41.9	40	53.4
1900	2920	18080	18099	53	50	5420	18696	18673	53.4	41.7	0.5	617	76.2	42.5	40	54.3
1901	2921	3095	3116	51.9	45.5	5421	3650	3631	53.1	50	1.2	556	76.2	42.8	40	54
1902	2922	18080	18099	53	50	5422	18696	18672	53.9	40	1	617	76.2	42.5	40	54.3
1903	2923	28868	28887	50.7	45	5423	29298	29279	52.6	55	1.9	431	76.8	45	40	54.1

1904	2924	3218	3238	52.1	47.6	5424	3650	3631	53.1	50	1	433	75.5	41.8	40	53.6
1905	2925	8867	8886	50.7	50	5425	9252	9234	51.4	52.6	0.8	386	75.1	41.5	40	52.9
1906	2926	28867	28887	53.7	47.6	5426	29306	29287	54.6	55	0.8	440	76.9	45.2	40	55.1
1907	2927	3218	3237	50.5	45	5427	3497	3478	51.3	50	0.8	280	74.6	41.8	40	52.5
1908	2928	29195	29215	53.2	47.6	5428	29306	29287	54.6	55	1.4	112	73.6	48.2	40	52.6
1909	2929	29196	29215	51.8	50	5429	29298	29279	52.6	55	0.8	103	73.3	48.5	40	51.9
1910	2930	3218	3237	50.5	45	5430	3500	3481	51.2	50	0.6	283	74.6	41.7	40	52.5
1911	2931	8867	8886	50.7	50	5431	9245	9226	50	45	0.6	379	75	41.2	40	52.6
1912	2932	28868	28888	51.4	42.9	5432	29298	29279	52.6	55	1.2	431	76.8	45	40	54.3
1913	2933	8867	8886	50.7	50	5433	9107	9086	51.6	45.5	0.9	241	74.1	41.5	40	52.2
1914	2934	28867	28888	54.3	45.5	5434	29306	29287	54.6	55	0.3	440	76.9	45.2	40	55.2
1915	2935	19906	19925	50.1	50	5435	20615	20597	50.6	47.4	0.5	710	75.5	40.3	40	53
1916	2936	16551	16568	51.1	50	5436	16775	16756	50.3	45	0.8	225	73.8	41.3	40	51.9
1917	2937	8861	8880	50.2	45	5437	9341	9322	51.1	50	0.9	481	75.5	41.6	40	53
1918	2938	16368	16387	50.2	45	5438	16781	16761	51.3	47.6	1	414	75	40.8	40	52.7
1919	2939	3055	3074	51.1	50	5439	3209	3189	50.5	47.6	0.6	155	74.1	45.2	40	52.1
1920	2940	3217	3236	51.1	50	5440	3650	3631	53.1	50	2	434	75.5	41.9	40	53.3
1921	2941	28868	28889	52	40.9	5441	29298	29279	52.6	55	0.6	431	76.8	45	40	54.5
1922	2942	28867	28889	54.8	43.5	5442	29306	29287	54.6	55	0.2	440	76.9	45.2	40	55.3
1923	2943	3404	3422	50.5	47.4	5443	3503	3484	51.5	50	0.9	100	71.6	45	40	50.4
1924	2944	16368	16387	50.2	45	5444	16777	16758	51.5	50	1.2	410	75	40.7	40	52.6
1925	2945	24029	24047	52.1	52.6	5445	24815	24792	53.4	41.7	1.3	787	76.3	42.1	40	54.1
1926	2946	16368	16387	50.2	45	5446	16711	16691	51	42.9	0.8	344	75.1	41.9	40	52.7
1927	2947	28867	28890	55.2	41.7	5447	29306	29287	54.6	55	0.6	440	76.9	45.2	40	55.3
1928	2948	29196	29214	51.1	52.6	5448	29298	29279	52.6	55	1.5	103	73.3	48.5	40	51.7
1929	2949	18488	18507	53.7	55	5449	19224	19200	52.4	40	1.3	737	76	41.5	40	54
1930	2950	28395	28413	50.2	42.1	5450	28506	28488	50.2	47.4	0	112	74.4	50	40	52.2
1931	2951	16551	16568	51.1	50	5451	17032	17011	52	45.5	0.9	482	75.8	42.1	40	53.5
1932	2952	28871	28891	50.9	42.9	5452	29358	29339	52.8	50	1.9	488	76.9	44.9	40	54.2
1933	2953	28871	28891	50.9	42.9	5453	29298	29280	51.4	52.6	0.5	428	76.8	45.1	40	54.2
1934	2954	28870	28891	52.2	40.9	5454	29306	29288	53.5	52.6	1.2	437	76.8	45.1	40	54.6
1935	2955	28868	28891	53.8	41.7	5455	29301	29282	55.3	55	1.5	434	76.9	45.2	40	55.1
1936	2956	3404	3422	50.5	47.4	5456	3504	3485	50.4	45	0.1	101	71.5	44.6	40	50.3
1937	2957	29195	29213	51.9	52.6	5457	29298	29279	52.6	55	0.7	104	73.1	48.1	40	51.9
1938	2958	28938	28956	50.8	47.4	5458	29298	29280	51.4	52.6	0.6	361	76.4	44.9	40	53.8
1939	2959	18488	18507	53.7	55	5459	19210	19191	52	50	1.7	723	76	41.6	40	53.9
1940	2960	3095	3116	51.9	45.5	5460	3647	3628	50.6	45	1.3	553	76.2	42.7	40	53.6
1941	2961	3214	3233	51.1	50	5461	3497	3478	51.3	50	0.2	284	74.7	41.9	40	52.7
1942	2962	24017	24039	53	43.5	5462	24815	24791	54.5	40	1.5	799	76.2	41.9	40	54.4
1943	2963	3095	3116	51.9	45.5	5463	3646	3625	52	40.9	0.1	552	76.1	42.6	40	54
1944	2964	18550	18571	50.4	40.9	5464	19215	19194	50.2	40.9	0.2	666	75.8	41.1	40	53.2
1945	2965	3214	3233	51.1	50	5465	3500	3481	51.2	50	0.1	287	74.7	41.8	40	52.7
1946	2966	18586	18603	50.4	44.4	5466	19224	19200	52.4	40	1.9	639	75.6	40.8	40	53.1
1947	2967	18586	18603	50.4	44.4	5467	19217	19196	50.2	40.9	0.2	632	75.6	41	40	53.1
1948	2968	18586	18603	50.4	44.4	5468	19215	19194	50.2	40.9	0.2	630	75.6	41	40	53.1
1949	2969	18590	18608	50.6	42.1	5469	19224	19200	52.4	40	1.8	635	75.6	40.9	40	53.2
1950	2970	15255	15273	50.3	52.6	5470	15767	15746	50.7	40.9	0.4	513	75.1	40.2	40	52.7
1951	2971	28942	28961	50.2	45	5471	29414	29395	50.5	50	0.3	473	76.8	44.6	40	53.9
1952	2972	18590	18608	50.6	42.1	5472	19217	19196	50.2	40.9	0.3	628	75.7	41.1	40	53.1
1953	2973	3055	3074	51.1	50	5473	3207	3187	50.5	47.6	0.6	153	74	45.1	40	52
1954	2974	18590	18608	50.6	42.1	5474	19215	19194	50.2	40.9	0.3	626	75.7	41.1	40	53.1

1955	2975	18591	18611	51.7	42.9	5475	19224	19200	52.4	40	0.7	634	75.7	41	40	53.6
1956	2976	18591	18611	51.7	42.9	5476	19217	19196	50.2	40.9	1.4	627	75.7	41.1	40	53.2
1957	2977	18591	18611	51.7	42.9	5477	19215	19194	50.2	40.9	1.4	625	75.7	41.1	40	53.2
1958	2978	28546	28565	52.2	50	5478	28672	28654	50.6	52.6	1.6	127	76.5	53.5	40	53.8
1959	2979	29191	29210	54.4	55	5479	29415	29395	53.4	52.4	1	225	75.7	45.8	40	54.1
1960	2980	7880	7900	50.3	42.9	5480	8190	8172	50.3	47.4	0	311	74.9	41.8	40	52.6
1961	2981	3167	3189	51.6	43.5	5481	3650	3631	53.1	50	1.5	484	75.8	42.1	40	53.6
1962	2982	28965	28984	52.9	55	5482	29306	29288	53.5	52.6	0.6	342	76.5	45.3	40	54.5
1963	2983	3166	3188	51.6	43.5	5483	3650	3631	53.1	50	1.5	485	75.8	42.3	40	53.7
1964	2984	8867	8887	52.3	47.6	5484	9101	9081	50.5	47.6	1.9	235	74.1	41.7	40	52.1
1965	2985	23843	23863	50.3	42.9	5485	24013	23995	50.3	47.4	0	171	73.7	43.3	40	51.8
1966	2986	3403	3421	53.1	52.6	5486	3503	3484	51.5	50	1.7	101	71.9	45.5	40	50.9
1967	2987	16549	16567	54.9	52.6	5487	16777	16756	53.4	45.5	1.5	229	74	41.5	40	52.9
1968	2988	8868	8889	50.4	40.9	5488	9109	9087	50.5	43.5	0.1	242	73.9	40.9	40	51.9
1969	2989	8861	8880	50.2	45	5489	9311	9292	50.7	50	0.6	451	75.3	41.2	40	52.9
1970	2990	8868	8889	50.4	40.9	5490	9257	9238	50.5	45	0.1	390	75	41	40	52.7
1971	2991	8868	8889	50.4	40.9	5491	9313	9294	50.4	50	0	446	75.4	41.5	40	53
1972	2992	23841	23859	50.5	52.6	5492	24013	23995	50.3	47.4	0.1	173	74	43.9	40	52
1973	2993	28548	28568	50.5	42.9	5493	28672	28654	50.6	52.6	0	125	76.2	52.8	40	53.6
1974	2994	8867	8888	52.7	45.5	5494	9310	9291	51.2	45	1.5	444	75.4	41.4	40	53.2
1975	2995	28968	28988	50.9	47.6	5495	29298	29279	52.6	55	1.8	331	76.2	44.7	40	53.7
1976	2996	19907	19926	52.1	55	5496	20615	20597	50.6	47.4	1.6	709	75.5	40.3	40	53.1
1977	2997	8861	8880	50.2	45	5497	9252	9235	50.1	50	0.1	392	75	41.1	40	52.6
1978	2998	19909	19929	50.7	52.4	5498	20615	20597	50.6	47.4	0.2	707	75.5	40.3	40	53.1
1979	2999	3361	3382	51.9	45.5	5499	3500	3481	51.2	50	0.7	140	74.1	46.4	40	52.3
1980	3000	18696	18715	51.7	50	5500	18881	18862	50.2	45	1.5	186	74.1	43.5	40	52.1
1981	3001	28968	28989	51.5	45.5	5501	29298	29279	52.6	55	1.1	331	76.2	44.7	40	53.9
1982	3002	19709	19730	51.3	40.9	5502	19923	19903	50.9	47.6	0.4	215	73.9	41.9	40	52.1
1983	3003	3361	3382	51.9	45.5	5503	3497	3478	51.3	50	0.6	137	74.1	46.7	40	52.4
1984	3004	3361	3384	53.7	41.7	5504	3495	3473	51.8	43.5	1.9	135	74	46.7	40	52.5
1985	3005	19709	19730	51.3	40.9	5505	19924	19905	50.1	50	1.2	216	73.9	41.7	40	51.8
1986	3006	16378	16397	50.4	45	5506	16711	16691	51	42.9	0.6	334	75.2	42.2	40	52.9
1987	3007	3361	3382	51.9	45.5	5507	3504	3485	50.4	45	1.5	144	74.3	46.5	40	52.2
1988	3008	18704	18724	50.8	47.6	5508	19406	19388	50.6	47.4	0.1	703	75.4	40.3	40	53.1
1989	3009	8868	8889	50.4	40.9	5509	9314	9295	51.1	50	0.7	447	75.5	41.6	40	53
1990	3010	3361	3382	51.9	45.5	5510	3503	3484	51.5	50	0.5	143	74.4	46.9	40	52.6
1991	3011	19709	19730	51.3	40.9	5511	19931	19912	50.9	55	0.4	223	74.2	42.2	40	52.3
1992	3012	16548	16566	54.9	52.6	5512	16777	16756	53.4	45.5	1.5	230	73.9	41.3	40	52.9
1993	3013	8868	8889	50.4	40.9	5513	9315	9296	50	45	0.4	448	75.4	41.5	40	52.9
1994	3014	22321	22341	51.6	42.9	5514	22460	22441	50.7	45	0.9	140	71.5	40	40	50.3
1995	3015	29182	29202	51.2	42.9	5515	29412	29393	50.3	45	0.9	231	75.4	45	40	53
1996	3016	22173	22193	51	42.9	5516	22460	22441	50.7	45	0.3	288	74.1	40.3	40	52.1
1997	3017	29181	29201	52.4	47.6	5517	29413	29393	51.1	42.9	1.3	233	75.5	45.1	40	53.3
1998	3018	18704	18724	50.8	47.6	5518	18881	18862	50.2	45	0.5	178	73.8	43.3	40	51.9
1999	3019	20751	20771	51.3	47.6	5519	21301	21278	51.3	41.7	0	551	75.5	41	40	53.3
2000	3020	29181	29200	50	45	5520	29412	29393	50.3	45	0.3	232	75.5	45.3	40	53
2001	3021	20751	20771	51.3	47.6	5521	21304	21283	50.5	40.9	0.8	554	75.5	41	40	53.1
2002	3022	29173	29197	54.2	40	5522	29415	29395	53.4	52.4	0.8	243	75.7	45.3	40	54.1
2003	3023	8867	8888	52.7	45.5	5523	9247	9226	52	45.5	0.7	381	75	41.2	40	53.2
2004	3024	8867	8888	52.7	45.5	5524	9255	9236	51.1	45	1.6	389	75	41.1	40	52.9
2005	3025	29178	29198	51.4	42.9	5525	29412	29393	50.3	45	1.1	235	75.5	45.1	40	53.1

2006	3026	3163	3185	53.6	47.8	5526	3650	3631	53.1	50	0.5	488	75.9	42.4	40	54.2
2007	3027	19800	19817	50.4	50	5527	20033	20016	50.4	50	0	234	74.9	43.6	41	52.7
2008	3028	8867	8886	50.7	50	5528	9376	9355	51	40.9	0.3	510	75.7	41.8	41	53.3
2009	3029	19800	19817	50.4	50	5529	19930	19910	50.6	47.6	0.2	131	72.6	43.5	41	51
2010	3030	24418	24439	52.9	45.5	5530	25082	25064	51.1	52.6	1.8	665	75.8	41.2	41	53.5
2011	3031	25771	25790	51.1	45	5531	26182	26161	51.2	40.9	0.1	412	74.8	40.3	41	52.8
2012	3032	12976	12994	50.3	47.4	5532	13326	13306	50.7	42.9	0.3	351	76.1	44.2	41	53.5
2013	3033	12976	12994	50.3	47.4	5533	13328	13307	51.2	45.5	0.9	353	76.1	44.2	41	53.5
2014	3034	2823	2844	50.4	45.5	5534	3500	3481	51.2	50	0.7	678	76.3	42.5	41	53.7
2015	3035	18009	18028	51.6	55	5535	18223	18205	53.3	52.6	1.7	215	74.5	43.3	41	52.7
2016	3036	8223	8240	50.4	50	5536	8933	8916	52.2	50	1.8	711	75.4	40.1	41	53
2017	3037	29180	29199	50.1	40	5537	29414	29395	50.5	50	0.4	235	75.5	45.1	41	53
2018	3038	19800	19817	50.4	50	5538	19925	19906	50.1	50	0.4	126	72.4	43.7	41	50.8
2019	3039	25772	25793	52.4	40.9	5539	26183	26162	52.8	45.5	0.4	412	74.8	40.3	41	53.2
2020	3040	14951	14975	52.2	40	5540	15152	15135	51.4	50	0.8	202	73.4	41.1	41	51.9
2021	3041	2823	2844	50.4	45.5	5541	3503	3484	51.5	50	1	681	76.4	42.6	41	53.7
2022	3042	18075	18095	50.6	47.6	5542	18231	18210	52.2	45.5	1.6	157	73.6	43.9	41	51.8
2023	3043	5	23	51.3	52.6	5543	269	251	51.1	52.6	0.1	265	76.4	46.4	41	53.9
2024	3044	9140	9159	50.1	45	5544	9249	9231	50.8	47.4	0.7	110	71.3	42.7	41	50
2025	3045	24418	24439	52.9	45.5	5545	24815	24791	54.5	40	1.6	398	75.9	43.2	41	54.1
2026	3046	8794	8813	51.6	45	5546	9358	9338	51	42.9	0.6	565	75.8	41.8	41	53.5
2027	3047	24418	24439	52.9	45.5	5547	24807	24786	51.7	45.5	1.2	390	75.9	43.3	41	53.8
2028	3048	2387	2405	51.6	52.6	5548	3186	3165	50.4	40.9	1.2	800	76.9	43.5	41	54.1
2029	3049	24418	24439	52.9	45.5	5549	24527	24506	51.7	40.9	1.2	110	71.3	42.7	41	50.5
2030	3050	24418	24439	52.9	45.5	5550	24517	24494	53.2	41.7	0.3	100	70.8	43	41	50.5
2031	3051	4255	4276	51.7	45.5	5551	4836	4817	51.2	45	0.5	582	75.9	41.9	41	53.6
2032	3052	24420	24440	50.8	42.9	5552	25082	25064	51.1	52.6	0.3	663	75.7	41	41	53.3
2033	3053	8867	8887	52.3	47.6	5553	9250	9232	51.6	47.4	0.8	384	75.1	41.4	41	53.2
2034	3054	14951	14975	52.2	40	5554	15275	15257	50.8	52.6	1.3	325	74.6	40.9	41	52.6
2035	3055	2387	2405	51.6	52.6	5555	3185	3164	51	45.5	0.7	799	76.9	43.6	41	54.2
2036	3056	8865	8884	50.4	45	5556	9252	9235	50.1	50	0.3	388	75.1	41.2	41	52.7
2037	3057	24420	24440	50.8	42.9	5557	24818	24797	51.6	40.9	0.8	399	75.8	42.9	41	53.4
2038	3058	24420	24440	50.8	42.9	5558	24807	24786	51.7	45.5	0.9	388	75.8	43	41	53.4
2039	3059	11541	11560	50.1	45	5559	12110	12090	51.1	42.9	1	570	75.9	41.9	41	53.3
2040	3060	2387	2405	51.6	52.6	5560	2672	2653	51.6	50	0	286	77	47.6	41	54.5
2041	3061	24420	24440	50.8	42.9	5561	24526	24506	50.3	42.9	0.5	107	70.8	42.1	41	49.8
2042	3062	11540	11557	50.4	50	5562	12110	12090	51.1	42.9	0.7	571	76	42	41	53.4
2043	3063	6263	6282	50.9	45	5563	6483	6463	50.2	42.9	0.7	221	73.7	41.2	41	51.8
2044	3064	24418	24440	55	47.8	5564	24815	24791	54.5	40	0.5	398	75.9	43.2	41	54.6
2045	3065	18075	18095	50.6	47.6	5565	18233	18214	52	50	1.4	159	74	44.7	41	52.1
2046	3066	18075	18095	50.6	47.6	5566	18233	18215	51.3	52.6	0.7	159	74	44.7	41	52.1
2047	3067	2429	2447	50.2	47.4	5567	3055	3036	50.6	50	0.4	627	76.3	42.6	41	53.6
2048	3068	19800	19818	52.1	52.6	5568	19917	19896	50.9	45.5	1.2	118	71.9	43.2	41	50.7
2049	3069	24481	24500	50.1	45	5569	24936	24919	51.8	50	1.7	456	75.7	42.1	41	53.1
2050	3070	276	294	50.5	47.4	5570	713	695	50.7	47.4	0.2	438	79.1	50.7	41	55.7
2051	3071	19801	19819	53.2	52.6	5571	19927	19908	52.1	55	1.1	127	72.7	44.1	41	51.6
2052	3072	19801	19819	53.2	52.6	5572	19925	19905	51.4	52.4	1.9	125	72.5	44	41	51.3
2053	3073	3800	3824	53.6	40	5573	4318	4294	54.4	40	0.8	519	75.3	40.8	41	53.9
2054	3074	11540	11557	50.4	50	5574	12258	12238	50.3	42.9	0.2	719	76.2	42	41	53.5
2055	3075	24482	24502	50.3	42.9	5575	24938	24921	50.4	50	0.1	457	75.6	41.8	41	53.1
2056	3076	24482	24502	50.3	42.9	5576	24807	24786	51.7	45.5	1.4	326	75.4	42.9	41	53

2057	3077	8867	8888	52.7	45.5	5577	9364	9346	53.9	52.6	1.2	498	75.8	42.2	41	54
2058	3078	24481	24502	51.5	45.5	5578	25080	25062	53.5	52.6	2	600	75.5	40.8	41	53.4
2059	3079	8865	8884	50.4	45	5579	9107	9086	51.6	45.5	1.2	243	74	41.2	41	52
2060	3080	8867	8888	52.7	45.5	5580	9313	9293	52.1	47.6	0.6	447	75.5	41.6	41	53.5
2061	3081	2427	2445	52.1	52.6	5581	3055	3036	50.6	50	1.5	629	76.4	42.8	41	53.7
2062	3082	2823	2844	50.4	45.5	5582	3504	3485	50.4	45	0.1	682	76.3	42.5	41	53.7
2063	3083	24483	24503	51	42.9	5583	25085	25068	50.3	50	0.6	603	75.4	40.6	41	53
2064	3084	15255	15273	50.3	52.6	5584	15649	15632	50.1	50	0.2	395	75.1	41.3	41	52.7
2065	3085	24483	24503	51	42.9	5585	25082	25064	51.1	52.6	0.1	600	75.5	40.8	41	53.3
2066	3086	8867	8886	50.7	50	5586	9375	9354	50.4	40.9	0.3	509	75.7	41.8	41	53.2
2067	3087	12976	12994	50.3	47.4	5587	13329	13308	50.5	40.9	0.2	354	76.1	44.1	41	53.4
2068	3088	24483	24503	51	42.9	5588	25081	25063	52.4	52.6	1.4	599	75.5	40.7	41	53.2
2069	3089	379	398	50.1	45	5589	941	922	50.5	50	0.4	563	78.7	48.8	41	55.2
2070	3090	24483	24503	51	42.9	5590	24936	24919	51.8	50	0.8	454	75.7	42.1	41	53.4
2071	3091	19802	19820	53	52.6	5591	19927	19908	52.1	55	0.8	126	72.8	44.4	41	51.7
2072	3092	9934	9953	50.7	50	5592	10670	10649	51.3	40.9	0.6	737	75.7	40.8	41	53.3
2073	3093	8866	8885	51.1	45	5593	9312	9293	50.6	45	0.5	447	75.4	41.4	41	53
2074	3094	19846	19866	51.2	42.9	5594	20033	20016	50.4	50	0.8	188	74.2	43.6	41	52.2
2075	3095	19848	19867	50.7	45	5595	20033	20016	50.4	50	0.3	186	74.1	43.5	41	52.1
2076	3096	9538	9558	50.9	42.9	5596	10017	9999	52.8	52.6	1.9	480	75.5	41.5	41	53.2
2077	3097	8220	8238	51.5	47.4	5597	8933	8916	52.2	50	0.7	714	75.4	40.1	41	53.3
2078	3098	9140	9159	50.1	45	5598	9249	9232	50	50	0.1	110	71.3	42.7	41	50
2079	3099	12976	12994	50.3	47.4	5599	13332	13312	50.9	47.6	0.6	357	76.2	44.3	41	53.5
2080	3100	15752	15772	50.8	47.6	5600	16174	16154	50.4	42.9	0.4	423	75.1	40.9	41	52.8
2081	3101	18074	18094	51.1	42.9	5601	18232	18212	50.6	47.6	0.5	159	73.7	44	41	51.9
2082	3102	24559	24579	52	52.4	5602	25081	25063	52.4	52.6	0.4	523	75.5	41.1	41	53.5
2083	3103	24559	24579	52	52.4	5603	25079	25061	52.7	52.6	0.7	521	75.5	41.3	41	53.6
2084	3104	3169	3191	52.1	47.8	5604	3650	3631	53.1	50	1	482	75.9	42.3	41	53.8
2085	3105	28117	28135	50.6	52.6	5605	28672	28654	50.6	52.6	0.1	556	80	52	41	56.3
2086	3106	1809	1829	50.6	42.9	5606	2103	2082	52	45.5	1.5	295	75.4	43.4	41	53
2087	3107	24559	24579	52	52.4	5607	24933	24913	51.1	42.9	0.8	375	75.6	42.7	41	53.4
2088	3108	1809	1829	50.6	42.9	5608	2113	2094	50.1	45	0.4	305	75.4	43.3	41	52.9
2089	3109	28116	28134	50.8	47.4	5609	28505	28487	50.2	47.4	0.6	390	79.4	51.8	41	55.8
2090	3110	1808	1828	50.6	42.9	5610	2103	2082	52	45.5	1.5	296	75.5	43.6	41	53.1
2091	3111	15951	15975	53.1	40	5611	16210	16192	54.3	52.6	1.2	260	74.3	41.5	41	53.1
2092	3112	8865	8884	50.4	45	5612	9341	9322	51.1	50	0.7	477	75.6	41.7	41	53.1
2093	3113	15	33	50.7	52.6	5613	642	622	51.6	47.6	0.9	628	79	49.2	41	55.6
2094	3114	8861	8880	50.2	45	5614	9107	9086	51.6	45.5	1.4	247	73.9	40.9	41	51.9
2095	3115	1808	1828	50.6	42.9	5615	2113	2094	50.1	45	0.4	306	75.5	43.5	41	53
2096	3116	24562	24580	50.1	52.6	5616	24933	24913	51.1	42.9	1.1	372	75.5	42.5	41	53
2097	3117	28116	28134	50.8	47.4	5617	28672	28654	50.6	52.6	0.2	557	80	51.9	41	56.2
2098	3118	24560	24580	51.3	52.4	5618	25081	25063	52.4	52.6	1.1	522	75.4	41	41	53.3
2099	3119	24560	24580	51.3	52.4	5619	25079	25061	52.7	52.6	1.4	520	75.5	41.2	41	53.3
2100	3120	16366	16384	50.3	52.6	5620	16775	16755	51.1	42.9	0.7	410	75.1	41	41	52.7
2101	3121	16366	16384	50.3	52.6	5621	16774	16754	50.4	42.9	0.1	409	75.1	41.1	41	52.8
2102	3122	24569	24590	56.6	54.5	5622	25089	25070	55.8	55	0.8	521	75.4	40.9	41	54.6
2103	3123	24569	24590	56.6	54.5	5623	25088	25069	55	50	1.6	520	75.3	40.8	41	54.3
2104	3124	24567	24590	57.8	54.2	5624	25095	25072	59.3	54.2	1.5	529	75.4	40.8	41	55.2
2105	3125	24568	24591	58.9	54.2	5625	25095	25072	59.3	54.2	0.4	528	75.3	40.7	41	55.5
2106	3126	24568	24591	58.9	54.2	5626	25091	25070	59.1	54.5	0.2	524	75.4	40.8	41	55.5
2107	3127	24568	24591	58.9	54.2	5627	25090	25069	58.3	54.5	0.6	523	75.4	40.9	41	55.4

2108	3128	16366	16384	50.3	52.6	5628	16774	16753	51.1	40.9	0.8	409	75.1	41.1	41	52.8
2109	3129	1806	1825	51.1	45	5629	2103	2082	52	45.5	1	298	75.5	43.6	41	53.3
2110	3130	8374	8395	52.4	45.5	5630	9107	9086	51.6	45.5	0.8	734	75.5	40.2	41	53.4
2111	3131	24622	24643	57.1	54.5	5631	24935	24913	56.1	47.8	1	314	74.5	40.8	41	54.1
2112	3132	9130	9150	51.3	42.9	5632	9358	9338	51	42.9	0.3	229	74.5	42.8	41	52.5
2113	3133	12936	12957	53.7	45.5	5633	13530	13511	55.6	55	1.9	595	77.4	45.4	41	55.4
2114	3134	8373	8391	50.7	47.4	5634	9107	9086	51.6	45.5	0.9	735	75.4	40.1	41	53.1
2115	3135	1352	1371	56.1	55	5635	1701	1678	54.3	41.7	1.8	350	76.7	45.7	41	55.1
2116	3136	8867	8886	50.7	50	5636	9342	9323	52.1	50	1.4	476	75.7	42	41	53.3
2117	3137	1352	1371	56.1	55	5637	1701	1677	54.7	40	1.4	350	76.7	45.7	41	55.2
2118	3138	9130	9150	51.3	42.9	5638	9249	9232	50	50	1.3	120	71.7	42.5	41	50.3
2119	3139	16861	16880	50.8	50	5639	17062	17045	50.2	50	0.6	202	74.8	44.6	41	52.5
2120	3140	9130	9150	51.3	42.9	5640	9249	9231	50.8	47.4	0.5	120	71.7	42.5	41	50.5
2121	3141	9130	9150	51.3	42.9	5641	9249	9230	51.5	45	0.2	120	71.7	42.5	41	50.7
2122	3142	8372	8390	50.7	47.4	5642	9060	9039	50.3	40.9	0.4	689	75.4	40.2	41	53
2123	3143	18074	18093	50.3	45	5643	18232	18212	50.6	47.6	0.3	159	73.7	44	41	51.8
2124	3144	2671	2692	52.1	40.9	5644	3193	3172	52.6	50	0.5	523	75.8	41.9	41	53.8
2125	3145	16562	16581	52.6	50	5645	17064	17045	51.4	50	1.2	503	75.8	42.1	41	53.6
2126	3146	2671	2692	52.1	40.9	5646	3193	3173	51.4	47.6	0.7	523	75.8	41.9	41	53.6
2127	3147	8372	8390	50.7	47.4	5647	9107	9086	51.6	45.5	0.9	736	75.5	40.2	41	53.1
2128	3148	12726	12746	51.3	47.6	5648	13321	13301	50.3	42.9	0.9	596	76.7	43.6	41	53.9
2129	3149	8867	8886	50.7	50	5649	9312	9293	50.6	45	0.1	446	75.4	41.5	41	53
2130	3150	16562	16580	51.9	52.6	5650	17062	17045	50.2	50	1.7	501	75.8	42.1	41	53.2
2131	3151	27377	27397	53.4	47.6	5651	27674	27653	52.5	40.9	0.9	298	74.3	40.6	41	52.8
2132	3152	16556	16573	50.3	50	5652	17111	17090	51.1	40.9	0.8	556	76.1	42.4	41	53.5
2133	3153	7815	7833	51.5	52.6	5653	8531	8512	52	45	0.5	717	75.7	40.7	41	53.5
2134	3154	3223	3241	50.2	52.6	5654	3494	3473	50.4	40.9	0.2	272	74.6	41.9	41	52.4
2135	3155	8372	8390	50.7	47.4	5655	9109	9087	50.5	43.5	0.1	738	75.4	40.1	41	53.1
2136	3156	3041	3065	57.7	48	5656	3650	3628	56.3	47.8	1.4	610	76.3	42.8	41	55.4
2137	3157	9569	9591	53	43.5	5657	10017	9999	52.8	52.6	0.3	449	75.4	41.4	41	53.7
2138	3158	3041	3065	57.7	48	5658	3649	3625	56.6	44	1.2	609	76.3	42.7	41	55.5
2139	3159	13176	13196	51.4	47.6	5659	13321	13301	50.3	42.9	1	146	73.3	43.8	41	51.5
2140	3160	16366	16385	52.9	55	5660	16775	16755	51.1	42.9	1.8	410	75.1	41	41	53
2141	3161	16366	16385	52.9	55	5661	16775	16754	51.7	40.9	1.1	410	75.1	41	41	53.2
2142	3162	16366	16385	52.9	55	5662	16774	16753	51.1	40.9	1.8	409	75.1	41.1	41	53
2143	3163	1402	1422	50.2	42.9	5663	2104	2084	50.6	42.9	0.4	703	76.7	43.2	41	53.8
2144	3164	1402	1422	50.2	42.9	5664	1697	1678	50.3	45	0.1	296	76	44.9	41	53.4
2145	3165	3055	3076	52.4	45.5	5665	3503	3484	51.5	50	1	449	76.1	43.2	41	53.8
2146	3166	15211	15230	50.2	45	5666	16001	15980	51.1	45.5	0.9	791	75.6	40.3	41	53.1
2147	3167	12267	12290	54.5	41.7	5667	12414	12392	53.9	43.5	0.6	148	72.2	41.2	41	51.8
2148	3168	8861	8880	50.2	45	5668	9256	9237	50.8	45	0.6	396	75	40.9	41	52.6
2149	3169	3049	3071	56.3	52.2	5669	3650	3628	56.3	47.8	0	602	76.4	42.9	41	55.4
2150	3170	3049	3071	56.3	52.2	5670	3648	3625	55.5	41.7	0.8	600	76.3	42.7	41	55.2
2151	3171	8861	8880	50.2	45	5671	9313	9294	50.4	50	0.3	453	75.3	41.3	41	52.9
2152	3172	12352	12375	52.9	41.7	5672	12911	12891	51.2	47.6	1.7	560	76.1	42.5	41	53.7
2153	3173	7965	7985	51.9	42.9	5673	8531	8512	52	45	0.2	567	75.1	40	41	53.2
2154	3174	18017	18036	54.8	55	5674	18233	18212	53.5	50	1.3	217	74.7	43.8	41	53.5
2155	3175	8867	8886	50.7	50	5675	9257	9238	50.5	45	0.2	391	75.1	41.2	41	52.8
2156	3176	3221	3239	51.5	52.6	5676	3494	3473	50.4	40.9	1.1	274	74.5	41.6	41	52.4
2157	3177	1402	1422	50.2	42.9	5677	1697	1677	51	42.9	0.8	296	76	44.9	41	53.4
2158	3178	1402	1422	50.2	42.9	5678	1697	1676	51.7	40.9	1.5	296	76	44.9	41	53.4
2159	3179	18011	18032	55.7	54.5	5679	18220	18201	56.1	55	0.4	210	74.5	43.3	41	53.9

2160	3180	12726	12746	51.3	47.6	5680	13329	13308	50.5	40.9	0.8	604	76.6	43.5	41	53.9
2161	3181	1402	1425	52.8	41.7	5681	1698	1678	51.7	42.9	1.1	297	76	44.8	41	53.8
2162	3182	18013	18032	52.2	55	5682	18223	18205	53.3	52.6	1.1	211	74.4	43.1	41	52.8
2163	3183	3777	3797	51.7	47.6	5683	4444	4424	50.6	42.9	1.1	668	75.6	40.7	41	53.2
2164	3184	3777	3797	51.7	47.6	5684	4445	4425	50.6	42.9	1.1	669	75.6	40.7	41	53.2
2165	3185	7876	7895	51.5	45	5685	8189	8170	50.6	50	0.9	314	75.1	42.4	41	52.9
2166	3186	18014	18032	51	52.6	5686	18229	18209	50.1	42.9	0.8	216	74.2	42.6	41	52.1
2167	3187	1402	1425	52.8	41.7	5687	1697	1677	51	42.9	1.8	296	76	44.9	41	53.6
2168	3188	1402	1425	52.8	41.7	5688	1697	1676	51.7	40.9	1.1	296	76	44.9	41	53.8
2169	3189	1402	1425	52.8	41.7	5689	1501	1481	51.2	42.9	1.6	100	72	46	41	50.9
2170	3190	12366	12384	51.7	52.6	5690	13155	13138	50.4	50	1.3	790	76.8	43.4	41	54
2171	3191	27361	27380	52.4	55	5691	27573	27552	52.3	40.9	0.1	213	75	44.6	41	53.3
2172	3192	18006	18028	54.5	52.2	5692	18220	18201	56.1	55	1.6	215	74.5	43.3	41	53.6
2173	3193	1442	1461	51.6	55	5693	1872	1854	53.2	52.6	1.6	431	76.2	43.6	41	53.9
2174	3194	27361	27380	52.4	55	5694	27567	27547	51.1	42.9	1.3	207	75.1	44.9	41	53
2175	3195	9131	9151	50.4	42.9	5695	9249	9230	51.5	45	1.2	119	71.8	42.9	41	50.5
2176	3196	3217	3236	51.1	50	5696	3504	3485	50.4	45	0.7	288	74.8	42	41	52.6
2177	3197	18011	18029	51.3	52.6	5697	18232	18212	50.6	47.6	0.7	222	74.8	43.7	41	52.6
2178	3198	3055	3074	51.1	50	5698	3503	3484	51.5	50	0.4	449	76.1	43.2	41	53.7
2179	3199	8866	8886	52.3	47.6	5699	9364	9346	53.9	52.6	1.6	499	75.8	42.1	41	53.9
2180	3200	16368	16387	50.2	45	5700	16774	16752	52.2	43.5	2	407	75	40.8	41	52.7
2181	3201	8859	8879	50	42.9	5701	9252	9235	50.1	50	0.1	394	75	41.1	41	52.6
2182	3202	9131	9151	50.4	42.9	5702	9249	9231	50.8	47.4	0.5	119	71.8	42.9	41	50.5
2183	3203	3217	3236	51.1	50	5703	3494	3473	50.4	40.9	0.7	278	74.6	41.7	41	52.4
2184	3204	8859	8879	50	42.9	5704	9341	9322	51.1	50	1.1	483	75.6	41.6	41	53
2185	3205	9131	9151	50.4	42.9	5705	9249	9232	50	50	0.3	119	71.8	42.9	41	50.4
2186	3206	8867	8886	50.7	50	5706	9248	9229	50.1	45	0.5	382	75.1	41.4	41	52.7
2187	3207	27366	27384	52.2	52.6	5707	27576	27555	51	40.9	1.2	211	74.8	44.1	41	52.7
2188	3208	1442	1461	51.6	55	5708	1879	1861	53	52.6	1.4	438	76.2	43.6	41	54
2189	3209	12366	12384	51.7	52.6	5709	12724	12705	52.4	55	0.7	359	75.6	42.9	41	53.5
2190	3210	12366	12384	51.7	52.6	5710	12498	12480	50	47.4	1.6	133	73	44.4	41	51.2
2191	3211	98	118	50.6	42.9	5711	713	695	50.7	47.4	0.1	616	79	49.4	41	55.6
2192	3212	12373	12391	50.8	47.4	5712	13155	13138	50.4	50	0.4	783	76.8	43.4	41	54
2193	3213	18011	18030	52.9	55	5713	18230	18209	51.3	45.5	1.6	220	74.5	43.2	41	52.7
2194	3214	1402	1426	54.1	40	5714	1700	1676	53.9	40	0.2	299	76	44.8	41	54.5
2195	3215	18011	18030	52.9	55	5715	18223	18205	53.3	52.6	0.5	213	74.4	43.2	41	53.1
2196	3216	1402	1426	54.1	40	5716	1698	1677	52.3	40.9	1.8	297	76	44.8	41	54
2197	3217	16463	16483	51.3	42.9	5717	17032	17011	52	45.5	0.7	570	76	42.1	41	53.7
2198	3218	18009	18030	54.6	54.5	5718	18220	18201	56.1	55	1.6	212	74.5	43.4	41	53.6
2199	3219	1402	1426	54.1	40	5719	1700	1678	52.9	43.5	1.3	299	76	44.8	41	54.2
2200	3220	9131	9151	50.4	42.9	5720	9358	9338	51	42.9	0.6	228	74.6	43	41	52.4
2201	3221	3055	3075	51.8	47.6	5721	3503	3484	51.5	50	0.3	449	76.1	43.2	41	53.8
2202	3222	18013	18031	50.6	52.6	5722	18232	18212	50.6	47.6	0	220	74.7	43.6	41	52.6
2203	3223	8794	8813	51.6	45	5723	9249	9230	51.5	45	0.1	456	75.4	41.4	41	53.4
2204	3224	8794	8813	51.6	45	5724	9249	9231	50.8	47.4	0.8	456	75.4	41.4	41	53.1
2205	3225	16549	16567	54.9	52.6	5725	17065	17045	53.1	47.6	1.9	517	76	42.4	41	54.2
2206	3226	8794	8813	51.6	45	5726	9249	9232	50	50	1.6	456	75.4	41.4	41	52.9
2207	3227	1402	1426	54.1	40	5727	2104	2082	53.5	43.5	0.6	703	76.7	43.2	41	54.8
2208	3228	9927	9946	51.3	50	5728	10356	10336	52.4	47.6	1.1	430	75.6	42.1	41	53.4
2209	3229	3219	3238	50.7	50	5729	3494	3473	50.4	40.9	0.3	276	74.5	41.7	41	52.4
2210	3230	16549	16567	54.9	52.6	5730	17033	17011	53.2	43.5	1.7	485	75.8	42.1	41	54.1

2211	3231	18014	18032	51	52.6	5731	18702	18685	50.2	50	0.8	689	76.2	42.2	41	53.5
2212	3232	8794	8813	51.6	45	5732	9333	9315	52.2	52.6	0.6	540	75.9	42	41	53.7
2213	3233	8867	8888	52.7	45.5	5733	9249	9229	53	47.6	0.2	383	75.2	41.5	41	53.5
2214	3234	18009	18028	51.6	55	5734	18229	18209	50.1	42.9	1.5	221	74.5	43	41	52.3
2215	3235	9633	9651	51	47.4	5735	10017	9999	52.8	52.6	1.8	385	75.6	42.6	41	53.3
2216	3236	9915	9935	51.8	47.6	5736	10449	10428	51.9	40.9	0.1	535	75.4	40.9	41	53.4
2217	3237	29259	29277	50.9	52.6	5737	29414	29395	50.5	50	0.3	156	74.5	46.2	41	52.4
2218	3238	8868	8889	50.4	40.9	5738	9317	9297	50.5	42.9	0.1	450	75.4	41.6	41	53
2219	3239	29257	29276	51.3	50	5739	29414	29395	50.5	50	0.8	158	74.6	46.2	41	52.5
2220	3240	13176	13196	51.4	47.6	5740	13332	13312	50.9	47.6	0.5	157	73.6	43.9	41	51.9
2221	3241	9918	9938	51.4	47.6	5741	10449	10428	51.9	40.9	0.5	532	75.4	40.8	41	53.3
2222	3242	13176	13196	51.4	47.6	5742	13856	13835	50.1	45.5	1.3	681	75.8	41.1	41	53.2
2223	3243	29253	29270	50	50	5743	29414	29395	50.5	50	0.5	162	75.2	47.5	41	52.8
2224	3244	13037	13058	54.8	50	5744	13530	13511	55.6	55	0.8	494	77.3	45.7	41	55.6
2225	3245	18009	18028	51.6	55	5745	18702	18685	50.2	50	1.5	694	76.3	42.4	41	53.6
2226	3246	24178	24197	50.3	40	5746	24938	24921	50.4	50	0.1	761	75.8	40.9	41	53.2
2227	3247	24174	24195	52.5	40.9	5747	24740	24717	52.5	41.7	0	567	76	42.2	41	54
2228	3248	7679	7698	50.6	50	5748	8054	8035	50.4	50	0.1	376	75.6	42.6	41	53.1
2229	3249	18005	18024	51.1	50	5749	18229	18209	50.1	42.9	1	225	74.4	42.7	41	52.2
2230	3250	24174	24195	52.5	40.9	5750	24933	24913	51.1	42.9	1.4	760	75.8	40.9	41	53.5
2231	3251	3016	3036	50.2	42.9	5751	3500	3481	51.2	50	0.9	485	76.3	43.3	41	53.6
2232	3252	28820	28838	53.7	52.6	5752	29306	29288	53.5	52.6	0.2	487	77.1	45.4	41	55.1
2233	3253	18005	18024	51.1	50	5753	18233	18214	52	50	0.9	229	74.9	43.7	41	52.8
2234	3254	3016	3036	50.2	42.9	5754	3503	3484	51.5	50	1.2	488	76.3	43.4	41	53.6
2235	3255	7723	7741	52.2	52.6	5755	8054	8035	50.4	50	1.7	332	75	41.9	41	52.8
2236	3256	29200	29224	54.2	40	5756	29358	29339	52.8	50	1.4	159	74.5	45.9	41	53.1
2237	3257	3016	3036	50.2	42.9	5757	3504	3485	50.4	45	0.1	489	76.3	43.4	41	53.6
2238	3258	985	1004	51.1	50	5758	1499	1482	50.1	50	1.1	515	76.5	43.7	41	53.7
2239	3259	8866	8885	51.1	45	5759	9257	9238	50.5	45	0.6	392	75	41.1	41	52.8
2240	3260	3016	3036	50.2	42.9	5760	3647	3628	50.6	45	0.4	632	76.4	42.9	41	53.7
2241	3261	13039	13058	51.8	50	5761	13749	13727	50.5	43.5	1.3	711	76.7	43.2	41	53.9
2242	3262	24096	24119	54.4	41.7	5762	24815	24792	53.4	41.7	1	720	75.8	41	41	54.2
2243	3263	17840	17859	50.8	45	5763	18229	18209	50.1	42.9	0.7	390	74.7	40.3	41	52.4
2244	3264	15255	15273	50.3	52.6	5764	15647	15628	51	45	0.7	393	75.1	41.2	41	52.7
2245	3265	988	1006	52.2	52.6	5765	1500	1482	50.6	47.4	1.6	513	76.5	43.7	41	53.8
2246	3266	24035	24053	52.2	52.6	5766	24527	24508	50.5	45	1.7	493	75.4	41.2	41	53
2247	3267	18616	18636	51.4	47.6	5767	19215	19194	50.2	40.9	1.1	600	75.7	41.2	41	53.1
2248	3268	8374	8393	51.2	45	5768	9101	9081	50.5	47.6	0.7	728	75.5	40.2	41	53.1
2249	3269	17840	17859	50.8	45	5769	18238	18219	50.3	45	0.5	399	75	40.9	41	52.7
2250	3270	24030	24047	50.7	50	5770	24526	24506	50.3	42.9	0.4	497	75.5	41.2	41	53
2251	3271	24030	24047	50.7	50	5771	24527	24507	51	42.9	0.3	498	75.4	41.2	41	53.1
2252	3272	17840	17859	50.8	45	5772	18239	18220	50	45	0.8	400	74.9	40.8	41	52.6
2253	3273	985	1008	56.1	50	5773	1626	1602	56.1	44	0	642	77.1	44.5	41	55.9
2254	3274	13039	13057	51.1	52.6	5774	13749	13727	50.5	43.5	0.6	711	76.7	43.2	41	53.9
2255	3275	29200	29223	53.7	41.7	5775	29358	29339	52.8	50	0.9	159	74.5	45.9	41	53.1
2256	3276	1046	1063	50.3	50	5776	1498	1481	51	50	0.7	453	76.4	43.9	41	53.7
2257	3277	24019	24039	50.1	42.9	5777	24527	24508	50.5	45	0.4	509	75.4	41.1	41	52.9
2258	3278	24014	24035	50.6	40.9	5778	24527	24508	50.5	45	0.1	514	75.5	41.2	41	53.1
2259	3279	1046	1063	50.3	50	5779	1497	1480	50.3	50	0.1	452	76.5	44	41	53.7
2260	3280	29201	29222	51	40.9	5780	29358	29339	52.8	50	1.9	158	74.3	45.6	41	52.4
2261	3281	18583	18603	54.8	47.6	5781	18696	18672	53.9	40	0.8	114	70.5	40.4	41	50.6

2262	3282	12977	12996	50.2	40	5782	13326	13306	50.7	42.9	0.4	350	76	44	41	53.4
2263	3283	23843	23863	50.3	42.9	5783	24088	24070	50.5	52.6	0.2	246	75.7	45.1	41	53.2
2264	3284	29200	29221	52.6	45.5	5784	29358	29339	52.8	50	0.2	159	74.5	45.9	41	53
2265	3285	23843	23863	50.3	42.9	5785	24091	24073	50.9	52.6	0.5	249	75.8	45.4	41	53.3
2266	3286	17792	17813	51.6	40.9	5786	18231	18210	52.2	45.5	0.6	440	75	40.5	41	53.1
2267	3287	23843	23863	50.3	42.9	5787	24094	24076	50.9	52.6	0.5	252	75.9	45.6	41	53.4
2268	3288	8374	8393	51.2	45	5788	9109	9087	50.5	43.5	0.6	736	75.4	40.1	41	53.1
2269	3289	17793	17813	50	42.9	5789	18223	18206	51.8	50	1.7	431	74.9	40.4	41	52.5
2270	3290	1046	1063	50.3	50	5790	1481	1463	50.5	47.4	0.2	436	76.2	43.6	41	53.6
2271	3291	23842	23862	50.9	47.6	5791	24093	24075	50.9	52.6	0	252	75.9	45.6	41	53.5
2272	3292	23842	23862	50.9	47.6	5792	24527	24507	51	42.9	0.1	686	76.1	41.8	41	53.6
2273	3293	2823	2844	50.4	45.5	5793	3082	3058	52.3	40	1.9	260	74.3	41.5	41	52.3
2274	3294	18550	18571	50.4	40.9	5794	19316	19295	50	40.9	0.4	767	75.5	40.3	41	53
2275	3295	23841	23860	52.1	55	5795	24527	24507	51	42.9	1.1	687	76.1	41.9	41	53.7
2276	3296	23841	23860	52.1	55	5796	24527	24508	50.5	45	1.6	687	76.1	41.9	41	53.5
2277	3297	17793	17813	50	42.9	5797	18233	18215	51.3	52.6	1.3	441	75.1	40.8	41	52.7
2278	3298	1	19	50.1	52.6	5798	269	251	51.1	52.6	1.1	269	76.4	46.5	41	53.6
2279	3299	23841	23859	50.5	52.6	5799	24094	24076	50.9	52.6	0.4	254	76.1	46.1	41	53.5
2280	3300	8908	8925	51.1	50	5800	9249	9231	50.8	47.4	0.2	342	75.1	41.8	41	52.9
2281	3301	8908	8925	51.1	50	5801	9249	9230	51.5	45	0.5	342	75.1	41.8	41	53
2282	3302	23841	23859	50.5	52.6	5802	24500	24481	50.1	45	0.4	660	76.1	42.1	41	53.4
2283	3303	23841	23859	50.5	52.6	5803	24526	24506	50.3	42.9	0.2	686	76.1	42	41	53.5
2284	3304	18225	18243	51.4	52.6	5804	18632	18611	50.2	40.9	1.2	408	75.7	42.6	41	53.2
2285	3305	3794	3812	52.9	52.6	5805	4318	4294	54.4	40	1.5	525	75.5	41.1	41	53.8
2286	3306	8908	8925	51.1	50	5806	9245	9226	50	45	1	338	74.9	41.4	41	52.5
2287	3307	17790	17811	51.6	40.9	5807	18231	18210	52.2	45.5	0.6	442	75	40.5	41	53.1
2288	3308	18077	18100	54.7	45.8	5808	18443	18424	55.9	55	1.3	367	75.8	43.3	41	54.6
2289	3309	23838	23857	50.4	50	5809	24527	24507	51	42.9	0.6	690	76	41.7	41	53.4
2290	3310	23838	23857	50.4	50	5810	24527	24508	50.5	45	0.1	690	76	41.7	41	53.4
2291	3311	23735	23752	51.2	50	5811	24013	23995	50.3	47.4	0.8	279	74.1	40.5	41	52.1
2292	3312	18080	18100	53.3	47.6	5812	18220	18202	54.8	52.6	1.5	141	73.1	44	41	52.3
2293	3313	18081	18100	51.7	50	5813	18223	18206	51.8	50	0.1	143	73.2	44.1	41	51.9
2294	3314	18081	18100	51.7	50	5814	18231	18210	52.2	45.5	0.5	151	73.6	44.4	41	52.1
2295	3315	18081	18100	51.7	50	5815	18233	18214	52	50	0.4	153	74	45.1	41	52.4
2296	3316	18081	18100	51.7	50	5816	18233	18215	51.3	52.6	0.4	153	74	45.1	41	52.3
2297	3317	8911	8928	51.9	50	5817	9252	9235	50.1	50	1.8	342	75	41.5	41	52.6
2298	3318	17791	17811	50	42.9	5818	18223	18206	51.8	50	1.7	433	74.9	40.4	41	52.5
2299	3319	8911	8928	51.9	50	5819	9249	9231	50.8	47.4	1	339	75	41.6	41	52.8
2300	3320	12352	12375	52.9	41.7	5820	12912	12892	53.6	52.4	0.8	561	76.2	42.6	41	54.3
2301	3321	8911	8928	51.9	50	5821	9249	9230	51.5	45	0.3	339	75	41.6	41	53
2302	3322	12352	12375	52.9	41.7	5822	12995	12976	51.1	45	1.8	644	76.4	42.9	41	53.9
2303	3323	17791	17811	50	42.9	5823	18233	18215	51.3	52.6	1.3	443	75.1	40.9	41	52.7
2304	3324	12977	12996	50.2	40	5824	13328	13307	51.2	45.5	1	352	76	44	41	53.4
2305	3325	12977	12996	50.2	40	5825	13329	13308	50.5	40.9	0.3	353	76	43.9	41	53.4
2306	3326	8911	8928	51.9	50	5826	9245	9226	50	45	1.8	335	74.8	41.2	41	52.5
2307	3327	8913	8931	55.5	52.6	5827	9252	9231	54	45.5	1.5	340	74.9	41.5	41	53.7
2308	3328	1402	1425	52.8	41.7	5828	1501	1480	51.9	40.9	0.9	100	72	46	41	51.1
2309	3329	24941	24960	52	50	5829	25646	25627	50.5	45	1.5	706	75.4	40.2	41	53
2310	3330	17608	17628	50.9	42.9	5830	17769	17749	50	42.9	0.9	162	72.4	40.7	41	50.8
2311	3331	24941	24960	52	50	5831	25404	25386	52.7	52.6	0.7	464	75.3	41.2	41	53.4
2312	3332	24941	24960	52	50	5832	25401	25383	50.6	47.4	1.4	461	75.2	41	41	53

2313	3333	24941	24960	52	50	5833	25400	25382	51.4	52.6	0.6	460	75.3	41.1	41	53.2
2314	3334	17608	17628	50.9	42.9	5834	18231	18210	52.2	45.5	1.2	624	75.2	40.1	41	53.1
2315	3335	18081	18099	51.2	52.6	5835	18232	18212	50.6	47.6	0.6	152	73.8	44.7	41	51.9
2316	3336	7725	7742	50	50	5836	7853	7833	50.7	47.6	0.7	129	71.2	40.3	41	49.9
2317	3337	8913	8931	55.5	52.6	5837	9252	9230	54.5	43.5	1	340	74.9	41.5	41	53.9
2318	3338	17608	17628	50.9	42.9	5838	18233	18215	51.3	52.6	0.4	626	75.3	40.3	41	53.1
2319	3339	19715	19735	52.5	47.6	5839	19931	19912	50.9	55	1.6	217	74	41.9	41	52.2
2320	3340	8913	8931	55.5	52.6	5840	9248	9226	54.7	47.8	0.7	336	74.9	41.4	41	53.9
2321	3341	18081	18099	51.2	52.6	5841	18642	18622	50.5	42.9	0.7	562	76.2	42.7	41	53.6
2322	3342	2823	2844	50.4	45.5	5842	3189	3168	51	45.5	0.5	367	75.6	42.8	41	53.2
2323	3343	19715	19735	52.5	47.6	5843	19927	19908	52.1	55	0.3	213	73.9	41.8	41	52.4
2324	3344	2823	2844	50.4	45.5	5844	3190	3169	50.7	45.5	0.2	368	75.6	42.7	41	53.1
2325	3345	28936	28956	55.2	52.4	5845	29306	29287	54.6	55	0.6	371	76.6	45.3	41	55.1
2326	3346	28936	28956	55.2	52.4	5846	29306	29285	56.7	54.5	1.6	371	76.6	45.3	41	55.3
2327	3347	28523	28544	51.6	40.9	5847	29298	29280	51.4	52.6	0.2	776	78.4	47.3	41	55.4
2328	3348	24180	24199	50.3	40	5848	24933	24913	51.1	42.9	0.9	754	75.8	40.8	41	53.2
2329	3349	19715	19735	52.5	47.6	5849	19925	19905	51.4	52.4	1.1	211	73.8	41.7	41	52.2
2330	3350	4645	4665	50.2	42.9	5850	4836	4817	51.2	45	0.9	192	75	45.3	41	52.6
2331	3351	18081	18099	51.2	52.6	5851	18702	18685	50.2	50	1	622	76.2	42.4	41	53.5
2332	3352	28522	28542	50.2	42.9	5852	29298	29280	51.4	52.6	1.2	777	78.4	47.2	41	55.1
2333	3353	24179	24199	52.7	42.9	5853	24740	24717	52.5	41.7	0.2	562	76	42.2	41	54
2334	3354	19715	19735	52.5	47.6	5854	19909	19885	52.5	40	0	195	73.3	41	41	52.1
2335	3355	1810	1830	51.2	42.9	5855	2103	2082	52	45.5	0.8	294	75.5	43.5	41	53.3
2336	3356	19716	19737	52.2	45.5	5856	19909	19885	52.5	40	0.3	194	73.1	40.7	41	51.9
2337	3357	19719	19739	50.6	42.9	5857	19909	19885	52.5	40	1.9	191	72.9	40.3	41	51.3
2338	3358	12977	12996	50.2	40	5858	13332	13312	50.9	47.6	0.6	356	76.1	44.1	41	53.4
2339	3359	19721	19745	52.3	40	5859	19909	19885	52.5	40	0.2	189	73	40.7	41	51.9
2340	3360	17608	17627	50.2	45	5860	17769	17749	50	42.9	0.2	162	72.4	40.7	41	50.8
2341	3361	17608	17627	50.2	45	5861	18231	18210	52.2	45.5	2	624	75.2	40.1	41	52.8
2342	3362	19794	19813	50	50	5862	20099	20078	50.5	40.9	0.5	306	74.4	40.8	41	52.2
2343	3363	4658	4677	50.5	50	5863	5306	5289	50.8	50	0.3	649	75.5	40.7	41	53.1
2344	3364	24179	24200	53.3	40.9	5864	24580	24560	51.3	52.4	2	402	74.6	40	41	52.7
2345	3365	19794	19813	50	50	5865	19925	19906	50.1	50	0.1	132	72.8	43.9	41	51.1
2346	3366	1046	1064	51.2	47.4	5866	1498	1481	51	50	0.2	453	76.4	43.9	41	53.9
2347	3367	1046	1064	51.2	47.4	5867	1497	1480	50.3	50	0.9	452	76.5	44	41	53.7
2348	3368	2133	2152	50.7	45	5868	2675	2656	50.4	50	0.3	543	76.8	44.2	41	54
2349	3369	28965	28984	52.9	55	5869	29298	29279	52.6	55	0.3	334	76.4	45.2	41	54.4
2350	3370	24378	24397	55	55	5870	24564	24542	55	47.8	0	187	73.6	42.2	41	53.1
2351	3371	25348	25366	51.2	47.4	5871	25651	25632	52.7	50	1.6	304	74.7	41.4	41	52.7
2352	3372	19794	19813	50	50	5872	19923	19903	50.9	47.6	0.9	130	72.7	43.8	41	51
2353	3373	28967	28987	51.6	52.4	5873	29358	29339	52.8	50	1.2	392	76.5	44.6	41	54.1
2354	3374	17608	17627	50.2	45	5874	18233	18215	51.3	52.6	1.1	626	75.3	40.3	41	52.9
2355	3375	29186	29206	51.3	42.9	5875	29358	29339	52.8	50	1.5	173	74.5	45.1	41	52.6
2356	3376	24379	24398	55	55	5876	25093	25074	54.6	55	0.4	715	75.9	41.4	41	54.6
2357	3377	3170	3191	50.9	45.5	5877	3646	3625	52	40.9	1.1	477	75.7	41.9	41	53.4
2358	3378	3170	3191	50.9	45.5	5878	3647	3628	50.6	45	0.3	478	75.7	42.1	41	53.3
2359	3379	9140	9159	50.1	45	5879	9249	9230	51.5	45	1.4	110	71.3	42.7	41	50
2360	3380	12976	12995	51.1	45	5880	13326	13306	50.7	42.9	0.4	351	76.1	44.2	41	53.6
2361	3381	12976	12995	51.1	45	5881	13328	13307	51.2	45.5	0.1	353	76.1	44.2	41	53.7
2362	3382	3168	3189	51	45.5	5882	3494	3473	50.4	40.9	0.5	327	75.1	42.2	41	52.8
2363	3383	19794	19813	50	50	5883	19917	19896	50.9	45.5	0.9	124	72.3	43.5	41	50.7

2364	3384	24379	24398	55	55	5884	24517	24494	53.2	41.7	1.8	139	72.7	43.2	41	52
2365	3385	24380	24399	55	55	5885	25093	25074	54.6	55	0.4	714	76	41.5	41	54.6
2366	3386	2823	2844	50.4	45.5	5886	3201	3183	50.6	52.6	0.2	379	75.8	43	41	53.3
2367	3387	3798	3819	54.2	50	5887	4318	4294	54.4	40	0.2	521	75.4	41.1	41	54.2
2368	3388	9139	9159	52.5	47.6	5888	9852	9829	53.1	45.8	0.6	714	75.4	40.1	41	53.6
2369	3389	9139	9159	52.5	47.6	5889	9852	9828	53.6	44	1.1	714	75.4	40.1	41	53.6
2370	3390	19795	19814	50.4	45	5890	19927	19908	52.1	55	1.7	133	72.7	43.6	41	51.1
2371	3391	19795	19814	50.4	45	5891	19924	19905	50.1	50	0.3	130	72.4	43.1	41	50.8
2372	3392	12976	12995	51.1	45	5892	13329	13308	50.5	40.9	0.6	354	76.1	44.1	41	53.5
2373	3393	2133	2152	50.7	45	5893	2672	2654	50.9	52.6	0.2	540	76.8	44.3	41	54.1
2374	3394	4593	4613	51.5	47.6	5894	4836	4817	51.2	45	0.3	244	75.3	44.3	41	53.2
2375	3395	1810	1830	51.2	42.9	5895	2113	2094	50.1	45	1.1	304	75.5	43.4	41	53
2376	3396	17036	17058	53.5	47.8	5896	17483	17465	54.4	52.6	0.9	448	75.3	41.3	41	53.9
2377	3397	1046	1064	51.2	47.4	5897	1481	1463	50.5	47.4	0.6	436	76.2	43.6	41	53.6
2378	3398	9055	9079	52.8	40	5898	9255	9236	51.1	45	1.8	201	73.5	41.3	41	51.9
2379	3399	12976	12995	51.1	45	5899	13332	13312	50.9	47.6	0.2	357	76.2	44.3	41	53.7
2380	3400	8865	8884	50.4	45	5900	9311	9292	50.7	50	0.3	447	75.4	41.4	41	53
2381	3401	25363	25381	51.1	52.6	5901	25651	25632	52.7	50	1.6	289	74.3	40.8	41	52.5
2382	3402	3168	3189	51	45.5	5902	3504	3485	50.4	45	0.6	337	75.3	42.4	41	52.9
2383	3403	25363	25381	51.1	52.6	5903	25649	25629	51.5	42.9	0.3	287	74.1	40.4	41	52.3
2384	3404	29182	29202	51.2	42.9	5904	29414	29395	50.5	50	0.7	233	75.5	45.1	41	53.1
2385	3405	3031	3051	51.3	52.4	5905	3497	3478	51.3	50	0.1	467	76.3	43.5	41	53.9
2386	3406	29172	29192	51.5	42.9	5906	29412	29393	50.3	45	1.1	241	75.6	45.2	41	53.2
2387	3407	12040	12057	50.6	50	5907	12412	12392	50	42.9	0.6	373	75.9	43.4	41	53.2
2388	3408	11543	11562	50.4	40	5908	12110	12090	51.1	42.9	0.7	568	75.9	41.9	41	53.4
2389	3409	16909	16928	50.8	45	5909	17038	17021	50.7	50	0.1	130	72.7	43.8	41	51.2
2390	3410	16909	16928	50.8	45	5910	17039	17022	51.4	50	0.6	131	72.6	43.5	41	51.2
2391	3411	18077	18097	51.5	47.6	5911	18233	18214	52	50	0.5	157	73.9	44.6	41	52.3
2392	3412	18077	18097	51.5	47.6	5912	18233	18215	51.3	52.6	0.2	157	73.9	44.6	41	52.2
2393	3413	9055	9079	52.8	40	5913	9252	9234	51.4	52.6	1.4	198	73.7	41.9	41	52.1
2394	3414	25676	25697	51.9	40.9	5914	25832	25810	53.6	47.8	1.7	157	72.1	40.1	41	51.1
2395	3415	2223	2244	51.4	45.5	5915	2676	2657	50.7	50	0.7	454	76.9	45.2	41	54.2
2396	3416	619	640	50.4	45.5	5916	1171	1153	50.4	47.4	0	553	77.9	46.8	41	54.7
2397	3417	11541	11561	50.9	42.9	5917	12110	12090	51.1	42.9	0.3	570	75.9	41.9	41	53.5
2398	3418	3360	3379	50.7	45	5918	3497	3478	51.3	50	0.6	138	74	46.4	42	52.1
2399	3419	19725	19745	50	42.9	5919	19921	19901	50.2	47.6	0.1	197	73.5	41.6	42	51.6
2400	3420	19720	19740	51.3	42.9	5920	19921	19901	50.2	47.6	1.1	202	73.4	41.1	42	51.5
2401	3421	3360	3379	50.7	45	5921	3500	3481	51.2	50	0.5	141	74	46.1	42	52.1
2402	3422	19717	19738	50.8	40.9	5922	19921	19901	50.2	47.6	0.6	205	73.4	41	42	51.5
2403	3423	24562	24580	50.1	52.6	5923	25209	25190	50.6	50	0.5	648	76.1	42	42	53.4
2404	3424	24559	24579	52	52.4	5924	24740	24717	52.5	41.7	0.5	182	76	48.4	42	53.9
2405	3425	3360	3379	50.7	45	5925	3504	3485	50.4	45	0.3	145	74.2	46.2	42	52.2
2406	3426	19716	19737	52.2	45.5	5926	19921	19900	51.8	45.5	0.4	206	73.5	41.3	42	52.1
2407	3427	3232	3251	50.3	50	5927	3494	3473	50.4	40.9	0.1	263	74.3	41.4	42	52.2
2408	3428	26039	26058	54	55	5928	26657	26636	52.6	45.5	1.5	619	75.4	40.5	42	53.7
2409	3429	3232	3251	50.3	50	5929	3504	3485	50.4	45	0.1	273	74.6	41.8	42	52.4
2410	3430	19715	19735	52.5	47.6	5930	19921	19900	51.8	45.5	0.7	207	73.7	41.5	42	52.2
2411	3431	26039	26058	54	55	5931	26653	26631	53.2	43.5	0.9	615	75.3	40.3	42	53.8
2412	3432	3229	3248	50.6	50	5932	3494	3473	50.4	40.9	0.2	266	74.3	41.4	42	52.3
2413	3433	3229	3248	50.6	50	5933	3504	3485	50.4	45	0.3	276	74.5	41.7	42	52.4
2414	3434	3225	3244	52.4	55	5934	3495	3473	51.8	43.5	0.6	271	74.7	42.1	42	52.9

2415	3435	3222	3241	52	50	5935	3650	3631	53.1	50	1.1	429	75.5	42	42	53.6
2416	3436	24559	24579	52	52.4	5936	25209	25190	50.6	50	1.3	651	76.1	42.1	42	53.6
2417	3437	6158	6178	51.3	42.9	5937	6289	6267	52.2	43.5	0.9	132	71.3	40.2	42	50.4
2418	3438	19709	19730	51.3	40.9	5938	19917	19896	50.9	45.5	0.3	209	73.7	41.6	42	52
2419	3439	3223	3241	50.2	52.6	5939	3497	3478	51.3	50	1.1	275	74.7	42.2	42	52.5
2420	3440	3223	3241	50.2	52.6	5940	3646	3625	52	40.9	1.8	424	75.4	41.7	42	53
2421	3441	3223	3241	50.2	52.6	5941	3647	3628	50.6	45	0.4	425	75.5	41.9	42	53
2422	3442	3217	3237	51.8	47.6	5942	3650	3631	53.1	50	1.3	434	75.5	41.9	42	53.5
2423	3443	9352	9372	51.3	42.9	5943	10014	9996	50.7	52.6	0.6	663	75.6	40.7	42	53.2
2424	3444	23733	23752	55.6	55	5944	24022	24003	55.5	55	0.1	290	74.5	41.4	42	53.9
2425	3445	26040	26061	56.4	54.5	5945	26661	26639	55.3	47.8	1.2	622	75.5	40.7	42	54.5
2426	3446	9918	9938	51.4	47.6	5946	10608	10589	51	50	0.4	691	75.8	41.1	42	53.4
2427	3447	7724	7742	51.4	52.6	5947	7843	7825	52.8	52.6	1.3	120	70.7	40	42	50
2428	3448	26040	26061	56.4	54.5	5948	26655	26631	56.2	48	0.2	616	75.4	40.6	42	54.8
2429	3449	28117	28135	50.6	52.6	5949	28506	28488	50.2	47.4	0.4	390	79.4	51.8	42	55.8
2430	3450	3217	3236	51.1	50	5950	3497	3478	51.3	50	0.2	281	74.7	42	42	52.7
2431	3451	3217	3236	51.1	50	5951	3500	3481	51.2	50	0.1	284	74.7	41.9	42	52.7
2432	3452	3165	3187	51.6	43.5	5952	3650	3631	53.1	50	1.5	486	75.8	42.2	42	53.6
2433	3453	19709	19730	51.3	40.9	5953	19925	19906	50.1	50	1.2	217	74	41.9	42	51.9
2434	3454	9927	9945	50.8	52.6	5954	10199	10180	51.5	45	0.7	273	75.3	43.6	42	53.1
2435	3455	9929	9946	50	50	5955	10670	10649	51.3	40.9	1.3	742	75.7	40.8	42	53.1
2436	3456	19709	19730	51.3	40.9	5956	19927	19908	52.1	55	0.9	219	74	42	42	52.3
2437	3457	9934	9953	50.7	50	5957	10356	10336	52.4	47.6	1.7	423	75.6	42.1	42	53.2
2438	3458	19709	19730	51.3	40.9	5958	19930	19910	50.6	47.6	0.7	222	74	41.9	42	52.1
2439	3459	3164	3186	51.6	43.5	5959	3650	3631	53.1	50	1.5	487	75.9	42.3	42	53.7
2440	3460	3089	3110	51.8	45.5	5960	3188	3166	51.6	43.5	0.2	100	72	46	42	51
2441	3461	18979	19000	51.6	45.5	5961	19215	19194	50.2	40.9	1.4	237	73.5	40.1	42	51.6
2442	3462	26421	26441	51.5	42.9	5962	26900	26882	51.5	52.6	0.1	480	77.5	46.2	42	54.8
2443	3463	26421	26441	51.5	42.9	5963	26828	26810	52.9	52.6	1.4	408	76.6	44.9	42	54.2
2444	3464	11540	11557	50.4	50	5964	11826	11802	51.3	40	0.8	287	74.4	41.1	42	52.3
2445	3465	26421	26441	51.5	42.9	5965	26695	26678	50.5	50	1	275	74.9	42.5	42	52.7
2446	3466	11540	11557	50.4	50	5966	11819	11798	50.3	40.9	0.1	280	74.3	41.1	42	52.2
2447	3467	11540	11557	50.4	50	5967	11817	11797	50.4	42.9	0.1	278	74.3	41	42	52.2
2448	3468	23841	23859	50.5	52.6	5968	24515	24494	50.4	40.9	0.1	675	76.1	41.9	42	53.5
2449	3469	3055	3077	52.8	43.5	5969	3495	3473	51.8	43.5	0.9	441	76	43.1	42	53.9
2450	3470	3795	3813	52.1	52.6	5970	4565	4542	53.9	41.7	1.8	771	75.6	40.3	42	53.6
2451	3471	11540	11560	53.2	47.6	5971	11984	11966	53	52.6	0.2	445	75.1	40.7	42	53.6
2452	3472	11541	11561	50.9	42.9	5972	12165	12147	51.2	47.4	0.4	625	75.7	41.3	42	53.4
2453	3473	3795	3815	54.6	52.4	5973	4318	4294	54.4	40	0.2	524	75.5	41.2	42	54.3
2454	3474	7723	7741	52.2	52.6	5974	7853	7833	50.7	47.6	1.5	131	71.3	40.5	42	50.2
2455	3475	3055	3075	51.8	47.6	5975	3504	3485	50.4	45	1.4	450	76.1	43.1	42	53.5
2456	3476	3055	3074	51.1	50	5976	3494	3473	50.4	40.9	0.7	440	76	43	42	53.4
2457	3477	26421	26441	51.5	42.9	5977	26651	26631	50.2	42.9	1.3	231	73.8	41.1	42	51.8
2458	3478	28109	28130	50.2	40.9	5978	28672	28654	50.6	52.6	0.4	564	79.9	51.6	42	56.1
2459	3479	3055	3074	51.1	50	5979	3504	3485	50.4	45	0.7	450	76.1	43.1	42	53.5
2460	3480	12232	12250	51.9	52.6	5980	12412	12392	50	42.9	1.9	181	73.2	41.4	42	51.3
2461	3481	3034	3053	50.3	50	5981	3210	3190	50.5	47.6	0.2	177	74.9	45.8	42	52.6
2462	3482	3034	3053	50.3	50	5982	3494	3473	50.4	40.9	0.1	461	76.1	43.2	42	53.5
2463	3483	3034	3053	50.3	50	5983	3504	3485	50.4	45	0.1	471	76.2	43.3	42	53.5
2464	3484	12236	12256	51.2	42.9	5984	12498	12480	50	47.4	1.1	263	74.6	42.2	42	52.4
2465	3485	12352	12375	52.9	41.7	5985	12724	12705	52.4	55	0.5	373	75.7	42.9	42	53.8

2466	3486	26421	26441	51.5	42.9	5986	26585	26567	51	47.4	0.5	165	72.2	40	42	50.9
2467	3487	3031	3051	51.3	52.4	5987	3503	3484	51.5	50	0.1	473	76.3	43.6	42	53.9
2468	3488	18704	18724	50.8	47.6	5988	19480	19459	50.3	40.9	0.4	777	75.5	40.2	42	53.1
2469	3489	3016	3036	50.2	42.9	5989	3646	3625	52	40.9	1.8	631	76.4	42.8	42	53.6
2470	3490	2823	2844	50.4	45.5	5990	3053	3034	50.3	50	0.2	231	74	41.6	42	52
2471	3491	12366	12384	51.7	52.6	5991	12994	12976	50.3	47.4	1.3	629	76.4	42.9	42	53.7
2472	3492	12366	12384	51.7	52.6	5992	12992	12974	51.2	52.6	0.5	627	76.5	43.1	42	54
2473	3493	2823	2844	50.4	45.5	5993	3056	3037	52.1	55	1.6	234	74.2	41.9	42	52.2
2474	3494	2522	2541	51.4	45	5994	2672	2654	50.9	52.6	0.5	151	75.3	48.3	42	53
2475	3495	2522	2541	51.4	45	5995	2675	2656	50.4	50	1	154	75.2	48.1	42	52.9
2476	3496	2429	2447	50.2	47.4	5996	3056	3037	52.1	55	1.9	628	76.3	42.7	42	53.6
2477	3497	2429	2447	50.2	47.4	5997	3190	3169	50.7	45.5	0.5	762	76.6	42.9	42	53.8
2478	3498	27436	27455	52.7	45	5998	27541	27521	51.7	47.6	1	106	72.1	45.3	42	51.1
2479	3499	2429	2447	50.2	47.4	5999	3192	3171	51.9	50	1.7	764	76.7	43.1	42	53.8
2480	3500	2427	2445	52.1	52.6	6000	3056	3037	52.1	55	0	630	76.4	42.9	42	54.2
2481	3501	27389	27407	50.6	47.4	6001	27541	27521	51.7	47.6	1.1	153	73.2	43.1	42	51.5
2482	3502	2427	2445	52.1	52.6	6002	3190	3169	50.7	45.5	1.4	764	76.7	43.1	42	54
2483	3503	18616	18636	51.4	47.6	6003	19316	19295	50	40.9	1.4	701	75.4	40.2	42	52.9
2484	3504	2377	2395	52.4	52.6	6004	2672	2653	51.6	50	0.8	296	77	47.3	42	54.5
2485	3505	18591	18611	51.7	42.9	6005	19216	19195	50.2	40.9	1.4	626	75.7	41.1	42	53.1
2486	3506	12366	12384	51.7	52.6	6006	12739	12718	51	40.9	0.7	374	75.6	42.8	42	53.3
2487	3507	2377	2395	52.4	52.6	6007	2672	2654	50.9	52.6	1.5	296	77	47.3	42	54.3
2488	3508	16982	17001	51.2	55	6008	17111	17090	51.1	40.9	0.1	130	74.6	48.5	42	52.6
2489	3509	2377	2395	52.4	52.6	6009	2675	2656	50.4	50	2	299	77	47.2	42	54.1
2490	3510	18590	18608	50.6	42.1	6010	19216	19195	50.2	40.9	0.3	627	75.6	41	42	53.1
2491	3511	2377	2395	52.4	52.6	6011	2891	2873	50.8	47.4	1.6	515	76.8	44.5	42	54.1
2492	3512	8220	8240	54	47.6	6012	8935	8917	54.5	52.6	0.4	716	75.4	40.1	42	54.1
2493	3513	12370	12388	50.1	47.4	6013	12998	12979	50.1	45	0.1	629	76.4	42.9	42	53.6
2494	3514	2223	2244	51.4	45.5	6014	2675	2656	50.4	50	1	453	77	45.3	42	54.1
2495	3515	2220	2239	51.3	45	6015	2672	2654	50.9	52.6	0.4	453	77	45.3	42	54.2
2496	3516	24418	24439	52.9	45.5	6016	24936	24919	51.8	50	1.1	519	76	42.4	42	53.8
2497	3517	18586	18603	50.4	44.4	6017	19216	19195	50.2	40.9	0.2	631	75.6	40.9	42	53.1
2498	3518	2220	2239	51.3	45	6018	2675	2656	50.4	50	0.8	456	76.9	45.2	42	54.1
2499	3519	1402	1422	50.2	42.9	6019	2153	2134	50.4	45	0.2	752	76.7	43.1	42	53.8
2500	3520	1356	1375	53.8	55	6020	2153	2133	52.1	42.9	1.7	798	76.9	43.5	42	54.5

Table 5: Primers

Forward primer SEQ ID NO & Co-ordinates		Reverse primer SEQ ID NO & Co-ordinates		T_M (FOR & REV) (°C)		Product length (bp)
6076	1-19	6171	199-183	50.1	50.3	199
6077	149-169	6172	334-315	51.5	52.4	186
6078	292-310	6173	560-541	50.8	51.1	269
6079	598-619	6174	749-731	52.6	50.6	152
6080	721-742	6175	930-912	50.4	50.3	210
6081	888-912	6176	1077-1058	52.8	51.2	190
6082	984-1003	6177	1149-1131	51.1	51.1	166

6083	1157-1175	6178	1479-1460	50.9	51.6	323
6084	1420-1441	6179	1700-1680	51.2	50.7	281
6085	1685-1707	6180	1834-1811	53.8	53.7	150
6086	1740-1764	6181	1987-1963	53.4	52.2	248
6087	2007-2025	6182	2251-2232	50.3	50.1	245
6088	2226-2245	6183	2385-2366	50.4	50.1	160
6089	2428-2446	6184	2749-2728	50.1	50.3	322
6090	2742-2763	6185	2893-2875	50.6	51.4	152
6091	2823-2844	6186	3082-3058	50.4	52.3	260
6092	3007-3031	6187	3185-3164	51.9	51.0	179
6093	3234-3254	6188	3497-3478	51.1	51.3	264
6094	3453-3476	6189	3647-3627	51.8	52.1	195
6095	3601-3622	6190	3877-3853	52.5	53.6	277
6096	4007-4027	6191	4158-4135	51.1	51.4	152
6097	4141-4165	6192	4316-4295	51.3	50.8	176
6098	4366-4387	6193	4567-4544	54.6	55.4	202
6099	4488-4508	6194	4708-4690	50.7	50.3	221
6100	4658-4677	6195	4994-4974	50.5	51.2	337
6101	4902-4922	6196	5115-5092	50.5	51.4	214
6102	5239-5260	6197	5450-5430	50.8	50.9	212
6103	5366-5389	6198	5560-5542	50.5	51.8	195
6104	5593-5612	6199	5860-5836	50.8	51.6	268
6105	6042-6062	6200	6291-6271	50.4	51.1	250
6106	6271-6291	6201	6483-6463	51.1	50.2	213
6107	7017-7040	6202	7171-7153	52.4	52.8	155
6108	7253-7272	6203	7504-7486	50.3	50.3	252
6109	7415-7434	6204	7677-7654	54.5	53.6	263
6110	7615-7635	6205	7821-7798	51.1	52.8	207
6111	7728-7746	6206	7936-7915	51.7	50.1	209
6112	7845-7867	6207	7994-7970	52.7	53.4	150
6113	8011-8029	6208	8189-8170	51.4	50.6	179
6114	8143-8166	6209	8300-8281	52.2	50.8	158
6115	8221-8239	6210	8388-8369	51.0	51.1	168
6116	8553-8575	6211	8931-8915	51.8	50.3	379
6117	8867-8886	6212	9254-9236	50.7	50.6	388

6118	9244-9267	6213	9597-9573	51.9	53.4	354
6119	9620-9640	6214	9990-9969	51.3	51.3	371
6120	10009-10027	6215	10188-10171	50.2	50.2	180
6121	10093-10113	6216	10244-10223	52.4	50.6	152
6122	10242-10265	6217	10608-10589	51.2	51.0	367
6123	10549-10571	6218	10783-10763	53.7	55.2	235
6124	10766-10785	6219	10930-10912	52.0	51.1	165
6125	11065-11085	6220	11305-11287	50.7	50.0	241
6126	11265-11287	6221	11429-11405	54.5	53.5	165
6127	11552-11571	6222	11730-11709	52.0	50.4	179
6128	11705-11726	6223	11869-11848	50.1	50.2	165
6129	11801-11824	6224	11984-11967	51.5	50.4	184
6130	12040-12058	6225	12254-12235	52.3	51.9	215
6131	12235-12253	6226	12406-12388	50.1	50.1	172
6132	12366-12384	6227	12730-12712	51.7	52.2	365
6133	12727-12748	6228	12994-12976	50.8	50.3	268
6134	12948-12966	6229	13224-13201	50.7	51.7	277
6135	13175-13196	6230	13324-13300	54.3	55.1	150
6136	13237-13258	6231	13545-13526	52.9	52.9	309
6137	13790-13810	6232	13963-13945	50.9	50.7	174
6138	14080-14098	6233	14280-14257	51.5	51.0	201
6139	14405-14427	6234	14561-14540	50.2	50.9	157
6140	14882-14906	6235	15046-15024	50.9	51.5	165
6141	14951-14976	6236	15145-15124	53.1	52.9	195
6142	15113-15134	6237	15275-15257	51.6	50.8	163
6143	15211-15230	6238	15383-15363	50.2	50.1	173
6144	15364-15387	6239	15528-15506	54.0	52.1	165
6145	15456-15477	6240	15605-15585	52.0	53.2	150
6146	15513-15532	6241	15897-15876	51.2	50.4	385
6147	15837-15856	6242	15999-15978	52.3	50.8	163
6148	16073-16096	6243	16301-16277	51.7	52.8	229
6149	16245-16266	6244	16404-16380	50.3	52.0	160
6150	16366-16385	6245	16515-16492	52.9	53.8	150
6151	16553-16571	6246	16777-16758	53.4	51.5	225
6152	16832-16852	6247	17026-17004	51.0	51.6	195

6153	16982-17001	6248	17359-17340	51.2	50.2	378
6154	17354-17372	6249	17511-17490	51.3	50.4	158
6155	17422-17443	6250	17573-17552	50.2	51.1	152
6156	17603-17623	6251	17769-17748	50.7	51.5	167
6157	17728-17746	6252	17883-17862	50.9	51.2	156
6158	18011-18030	6253	18163-18140	52.9	51.9	153
6159	18076-18098	6254	18225-18205	54.4	55.0	150
6160	18270-18292	6255	18432-18413	51.9	51.4	163
6161	18352-18373	6256	18648-18629	51.3	50.8	297
6162	18550-18571	6257	18702-18684	50.4	51.9	153
6163	18720-18738	6258	19004-18983	50.6	51.0	285
6164	18960-18981	6259	19109-19085	54.7	54.3	150
6165	19065-19089	6260	19217-19195	52.8	51.7	153
6166	19310-19329	6261	19476-19454	50.2	52.1	167
6167	19569-19589	6262	19719-19701	50.5	51.8	151
6168	19707-19731	6263	19856-19833	55.7	55.9	150
6169	19771-19792	6264	19921-19901	50.1	50.2	151
6170	19833-19851	6265	19986-19966	50.9	50.7	154

Table 6: Primers

Forward primer SEQ ID NO & Co-ordinates		Reverse primer SEQ ID NO & Co-ordinates		T_M (FOR & REV) (°C)		Product length (bp)
6266	20110-20132	6305	20425-20404	51.9	50.9	316
6267	20468-20492	6306	20617-20596	53.2	53.5	150
6268	20557-20578	6307	20891-20871	50.4	50.6	335
6269	20838-20856	6308	21037-21015	52.5	52.0	200
6270	21096-21116	6309	21295-21272	50.1	51.7	200
6271	22173-22194	6310	22414-22395	52.4	51.0	242
6272	22320-22342	6311	22501-22479	54.8	54.3	182
6273	22532-22552	6312	22695-22675	50.6	50.0	164
6274	22712-22736	6313	22873-22852	56.7	55.5	162
6275	22842-22861	6314	23086-23067	51.0	52.8	245
6276	23151-23170	6315	23395-23376	51.4	50.3	245
6277	23307-23326	6316	23524-23501	51.1	51.1	218
6278	23615-23635	6317	23776-23758	50.7	50.2	162

6279	23838-23857	6318	23996-23977	50.4	50.6	159
6280	24030-24051	6319	24407-24386	57.6	55.7	378
6281	24388-24407	6320	24581-24563	50.4	50.1	194
6282	24559-24579	6321	24938-24921	52.0	50.4	380
6283	24922-24941	6322	25184-25166	50.1	51.2	263
6284	25201-25220	6323	25400-25382	51.1	51.4	200
6285	25363-25381	6324	25646-25627	51.1	50.5	284
6286	25656-25681	6325	25839-25814	54.5	56.4	184
6287	25761-25782	6326	25982-25961	54.6	54.3	222
6288	26039-26058	6327	26189-26166	54.0	53.0	151
6289	26184-26205	6328	26333-26310	50.9	51.8	150
6290	26422-26442	6329	26660-26641	51.3	50.2	239
6291	26571-26589	6330	26739-26715	51.7	53.2	169
6292	26733-26752	6331	26960-26941	51.1	52.2	228
6293	26866-26885	6332	27139-27117	50.7	51.9	274
6294	27300-27321	6333	27458-27439	51.2	50.2	159
6295	27361-27380	6334	27579-27558	52.4	51.1	219
6296	27718-27740	6335	27917-27901	50.7	50.0	200
6297	28041-28059	6336	28207-28189	50.8	50.8	167
6298	28166-28189	6337	28411-28393	52.2	52.9	246
6299	28395-28414	6338	28671-28653	51.5	50.2	277
6300	28654-28672	6339	28821-28800	50.6	52.3	168
6301	28867-28885	6340	29184-29166	51.5	51.6	318
6302	29183-29204	6341	29360-29342	50.4	50.4	178
6303	29262-29279	6342	29626-29606	50.1	50.2	365
6304	29538-29559	6343	29690-29670	50.0	50.4	153

Table 7: Primers

Name	SEQ ID NO:	Co-ordinates		Name	SEQ ID NO:	Co-ordinates
AB4f	6344	19869-19888		CB1r	6367	28011-28030
AB5f	6345	20238-20257		CB2r	6368	27671-27690
BC1f	6346	20581-20600		CB3r	6369	27301-27320
BC2f	6347	20950-20969		CB4r	6370	26931-26950
BC3f	6348	21339-21358		CB5r	6371	26575-26594
BC4f	6349	21708-21727		CB6r	6372	26191-26210
BC5f	6350	22041-22060		CB7r	6373	25841-25860
BC6f	6351	22410-22429		CB8r	6374	25476-25495
BC7f	6352	22759-22778		CB9r	6375	25126-25145

BC8f	6353	23131-23150		CB10r	6376	24791-24810
BC9f	6354	23500-23519		CB11r	6377	24422-24441
BC10f	6355	23841-23860		CB12r	6378	24031-24050
BC11f	6356	24210-24229		CB13r	6379	23673-23692
BC12f	6357	24560-24579		CB14r	6380	23298-23317
BC13f	6358	24941-24960		CB15r	6381	22928-22947
BC14f	6359	25310-25329		CB16r	6382	22567-22586
BC15f	6360	25675-25694		CB17r	6383	22196-22215
BC16f	6361	26044-26063		CB18r	6384	21831-21850
BC17f	6362	26413-26432		CB19r	6385	21431-21450
BC18f	6363	26763-26782		CB20r	6386	21073-21092
BC19f	6364	27132-27151		CB21r	6387	20715-20734
BC20f	6365	27491-27510		BA1r	6388	20345-20364
BC21f	6366	27845-27864		BA2r	6389	19969-19988
				BA3r	6390	19599-19618
				BA4r	6391	19228-19247
				BA5r	6392	18852-18871

Table 8: Primers

Name	SEQ ID NO	Co-ordinates	Name	SEQ ID NO	Co-ordinates
F1	6393	1-19	R1	6441	334-315
F2	6394	292-310	R2	6442	749-731
F3	6395	721-742	R3	6443	1077-1058
F4	6396	984-1003	R4	6444	1479-1460
F5	6397	1420-1441	R5	6445	1834-1811
F6	6398	1740-1764	R6	6446	2251-2232
F7	6399	2226-2245	R7	6447	2749-2728
F8	6400	2742-2763	R8	6448	3082-3058
F9	6401	3007-3031	R9	6449	3497-3478
F10	6402	3453-3476	R10	6450	3877-3853
F11	6403	4007-4027	R11	6451	4316-4295
F12	6404	4366-4387	R12	6452	4708-4690
F13	6405	4658-4677	R13	6453	5115-5092
F14	6406	5239-5260	R14	6454	5560-5542
F15	6407	5593-5612	R15	6455	6291-6271
F16	6408	6271-6291	R16	6456	7171-7153
F17	6409	7253-7272	R17	6457	7677-7654
F18	6410	7615-7635	R18	6458	7936-7915
F19	6411	7845-7867	R19	6459	8189-8170
F20	6412	8143-8166	R20	6460	8388-8369
F21	6413	8553-8575	R21	6461	9254-9236
F22	6414	9244-9267	R22	6462	9990-9969
F23	6415	10009-10027	R23	6463	10244-10223
F24	6416	10242-10265	R24	6464	10783-10763
F25	6417	10766-10785	R25	6465	11305-11287
F26	6418	11265-11287	R26	6466	11730-11709
F27	6419	11705-11726	R27	6467	11984-11967
F28	6420	12040-12058	R28	6468	12406-12388
F29	6421	12366-12384	R29	6469	12994-12976
F30	6422	12948-12966	R30	6470	13324-13300
F31	6423	13237-13258	R31	6471	13963-13945

F32	6424	14080-14098	R32	6472	14561-14540
F33	6425	14882-14906	R33	6473	15145-15124
F34	6426	15113-15134	R34	6474	15383-15363
F35	6427	15364-15387	R35	6475	15605-15585
F36	6428	15513-15532	R36	6476	15999-15978
F37	6429	16073-16096	R37	6477	16404-16380
F38	6430	16366-16385	R38	6478	16777-16758
F39	6431	16832-16852	R39	6479	17359-17340
F40	6432	17354-17372	R40	6480	17573-17552
F41	6433	17603-17623	R41	6481	17883-17862
F42	6434	18011-18030	R42	6482	18225-18205
F43	6435	18270-18292	R43	6483	18648-18629
F44	6436	18550-18571	R44	6484	19004-18983
F45	6437	18960-18981	R45	6485	19217-19195
F46	6438	19310-19329	R46	6486	19719-19701
F47	6439	19707-19731	R47	6487	19921-19901
F48	6440	19833-19851			

Table 9: Primers

	Name	SEQ ID NO:
1	CB12R	6488
2	R0010	6489
3	R0011	6490
4	R0012	6491
5	BNI-ED	6492
6	BNI-EU	6493
7	SAR1S-U	6494
8	SAR1As-D	6495
9	SAR1S	6496
10	SAR1As	6497
11	IN2-U	6498
12	IN4-D	6499
13	IN-2	6500
14	IN-4	6501
15	IN-6	6502
16	IN-7	6503
17	COR1-U	6504
18	COR2-D	6505
19	COR-1	6506
20	COR-2	6507
21	HKUF-U	6508
22	HKUR-D	6509
23	HKU-F	6510
24	HKU-R	6511
25	1451-D	6512
26	1451-U	6513
27	690-D	6514
28	690-U	6515
29	690-D2	6516

	Name	SEQ ID NO:
37	EMC8-D2	6524
38	EMC8-U2	6525
39	EMC11-D	6526
40	EMC11-U	6527
41	ORF1B-D	6528
42	ORF1B-U	6529
43	ORFS-D	6530
44	ORFS-U	6531
45	E7-717F	6532
46	E8-85R	6533
47	E8-307F	6534
48	E11-771F	6535
49	E11-96R	6536
50	CON1-F	6537
51	CON1-U	6538
52	CON2-F	6539
53	CON2-R	6540
54	CON3-F	6541
55	CON3-R	6542
56	15-F	6543
57	15-R	6544
58	15-F2	6545
59	15-R2	6546
60	13-F	6547
61	13-R	6548
62	13-F2	6549
63	13-R2	6550
64	CONTIG-F	6551
65	QT3-R	6552

30	690-U2	6517
31	EMC7-D	6518
32	EMC7-U	6519
33	EMC7-D2	6520
34	EMC7-U2	6521
35	EMC8-D	6522
36	EMC8-U	6523

66	QT3-F	6553
67	QIN-R	6554
68	QIN-F	6555
69	AB1-F	6556
70	AB2-F	6557
71	AB3-F	6558
72	AB1-R	6559

Table 10: Features of the predicted proteins and open reading frames of the SARS virus

	SARS ORF (SEQ ID NO)	Length (aa)	Role	Cleavage site	Features	Cons[d] *
ORF1a	P28 (9766)	179	Leader protein	179 (G/G)[#]		+
	P65 (9767)	639	Homologue of MHV p65 cleavage product	818 (G/A)		+
	Nsp1 (9768)	2422 [##]	Papain like protease, cleaves the first two proteins	3240 (Q/S)	phosphoesterase domain Zn binding domain	+
	Nsp2 (9769)	306	3C-like protease, cleaves proteins nsp1-nsp12	3546 (Q/G)		+
	Nsp3 (9770)	290	?	3836 (Q/S)	5 TMDs	+
	Nsp4 (9771)	83	?	3919 (Q/A)	1 TMD	+
	Nsp5 (9772)	198	?	4117 (Q/N)		+
	Nsp6 (9773)	113	?	4230 (Q/A)		+
	Nsp7 (9774)	139	?	4369 (Q/S)	Putative growth factor-like motif	+
ORF1b	Nsp9 (9775)	932	RNA polymerase	5298 (Q/A)		+
	Nsp10 (9776)	601	Putative helicase Tanner et al. (2003) *J Biol Chem* 278:39578-82	5899 (Q/A)	Metal binding domain, ATP/GTP binding domain	+
	Nsp11 (9777)	527	?	6426 (Q/S)		+
	Nsp12 (9778)	346	?	6772 (Q/A)		+
	Nsp13 (9779)	298	?	-		+
Structural region	Spike (S) (6042)		Major antigenic determinant, contains the receptor-binding domain		Leader peptide, 1 TMD, 17 N-glycosylation sites	+
	Orf3 (6043)	274	?		2 TMDs, 1 N-glycosylation site, 10 O-glycosylation sites	-
	Orf4 (6044)	154	?			-
	Envelope (E) (6045)	76	Associated with viral envelope		1 TMD, 2 N-glycosylation sites	+
	Matrix (M) (6046)	221	Associated with viral envelope, membrane spanning protein		3 TMDs, 1 N-glycosylation site	+
	Orf7 (6047)	63	?		1 TMD	-
	Orf8 (6048)	122	?		1 TMD	-
	Orf9 (6049)	44	?		Surface-associated	-
	Orf10	39	?		Surface-associated	-
	Orf11(6050)	84	?		1 N-glycosylation site	-
	Nucleocapsid (N) (6052)	422	Associated with viral genomic RNA		phosphoprotein	+
	Orf13	98	?		1 O-glycosylation site	-

TMD: predicted transmembrane domain.
Cons[d] *: + indicates presence of corresponding protein at least in one of the other coronaviruses
#: Alternatively, cleaved after Gly-Gly (*i.e.* at G/A) to give a 180mer
##: This 2422mer may be further cleaved after residue 1922 (Gly-2740 of SEQ ID NO: 6039) to give a 1922mer PLpro containing the Zn-binding motif (SEQ ID NO: 7254) and a 500mer.

Table 11: Protein homologies between SARS and other coronaviruses

Numbers indicate percentage of aminoacid identity between SARS proteins and corresponding gene products of other coronaviruses. More conserved pairs are in bold; more variable pairs are underlined.

	group 1			group 2		group 3
Proteins	229E	TGV	PEDV	MHV	BCoV	AIBV
REPLICASE REGION						
leader protein p28	<20	<20	<20	**27**	<20	<20
p65 homologue	<20	**23**	23	<20	20	<20
nsp1 (PLP protease)	25.5	25.8	25.4	29	**30**	25
nsp2 (3CL protease)	40.4	43.8	44.6	**50**	48.4	41
nsp3	30	27	29.4	34.2	**35.5**	28.5
nsp4	38.6	42.2	39.8	**47.5**	46.1	37.3
nsp5	**48.2**	42.9	43.9	46.8	47.3	38.7
nsp6	45.1	38.9	45.1	45.1	**46.9**	39.8
nsp7	53.8	54.5	56.1	56.2	55.4	**58.3**
nsp9 (polymerase)	59.8	59.6	60	**67.3**	66.9	62.4
nsp10 (helicase)	60.7	62	62.3	67.2	68.6	58.9
nsp11	52.3	53.7	52.3	**57.6**	57.6	52
nsp12	43.1	43	45.4	**45.9**	45	40.2
nsp13	56.4	54.4	55.3	63	**65**	53.4
STRUCTURAL REGION						
Spike (S)	28.8	31.6*	30.3	**31.1**	31	32.7*
Envelope (E)	33*	**27.9**	20	23	26.5	23.2
Matrix glycoprotein (M)	30.6	32.5	34.8	40.8	**41.9**	32.5
Nucleocapsid (N)	26.9	30.1	29.5	37.3	**37.4**	31.5

* These three alignments were obtained only on a fragment of the whole protein.

Table 12: Nucleotide and aminoacid differences between five SARS isolates

		FRA*	TOR2*	Urbani*	CUHK*	HKU*
	position°	base/aminoacid	base/aminoacid	base/aminoacid	base/aminoacid	base/aminoacid
ORF1a	2557	A/Thr	G/Ala	G/Ala	G/Ala	G/Ala
	2601	T/Val				C
	7746	G/Pro			T	
	7919	C/Ala		T/Val		
	7930	G/Asp				A/Asn
	8387	G/Ser				C/Thr
	8416	G/Arg				C/Thr
	9404	T/Val			C/Ala	
	9479	T/Val			C/Ala	
	11448	T/Ile	C	C	C	C
ORF1b	13494	GT/Val				AG/Ser
	16622	C/Ala		T		
	17564	T/Asp			C/Glu	
	17846	C/Arg			T	
	18065	G/Lys				A
	18965	A/Ile	T	T	T	T
	19064	A/Glu		G	G	
	19084	T/Ile	C/Thr	C/Thr	C/Thr	C/Thr
spike	21721	G/Gly			A/Asp	
	22222	T/Ile			C/Thr	
	23220	T/Ser	G/Ala			
	24872	T/Leu		C		
	24933	T/Phe	C/Leu	C/Leu	C/Leu	C/Leu
ORF3	25298	G/Gly	A/Arg			
	25569	T/Met				A/Lys
matrix	26600	T/Val	C/Ala	C/Ala	C/Ala	
	26857	T/Ser		C/Pro		
ORF10	27827	T/Cys			C/Arg	
nucleocapsid	28268	T/Ile	C/Thr	C/Thr	C/Thr	C/Thr

* SARS coronavirus FRA (accession number AY310120)
SARS coronavirus TOR2 (accession number AY274119)
SARS coronavirus Urbani (accession number AY278741)
SARS coronavirus CUHK-W1 (accession number AY278554)
SARS coronavirus HKU-39849 (accession number AY278491)

° The position is based on the FRA sequence.

TABLES 13-25: T-epitope predictions for SEQ ID NOS: 6039-6050 & 6052

Epitope predictions were performed at *http://www.mpiib-berlin.mpg.de/MAPPP/binding.html* using a minimum score of 0.5 and the BIMAS matrix, with a maximum of 20 results being selected. The analysis revealed 9mer and 10mer epitopes.

Table 13: Epitopes for SEQ ID NO: 6039

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1867	SEQ ID NO: 7400	8 %	450
2	4139	SEQ ID NO: 7401	5.55 %	312.5
3	88	SEQ ID NO: 7402	4 %	225
4	4249	SEQ ID NO: 7403	3.55 %	200
5	4059	SEQ ID NO: 7404	2.22 %	125
6	2027	SEQ ID NO: 7405	1.6 %	90
7	3413	SEQ ID NO: 7406	1.11 %	62.5
8	1823	SEQ ID NO: 7407	0.88 %	50
9	2798	SEQ ID NO: 7408	0.88 %	50
10	220	SEQ ID NO: 7409	0.8 %	45
11	3738	SEQ ID NO: 7410	0.8 %	45
12	4182	SEQ ID NO: 7411	0.8 %	45
13	4174	SEQ ID NO: 7412	0.66 %	37.5
14	1940	SEQ ID NO: 7413	0.55 %	31.25
15	38	SEQ ID NO: 7414	0.48 %	27
16	1231	SEQ ID NO: 7415	0.44 %	25
17	1613	SEQ ID NO: 7416	0.44 %	25
18	3645	SEQ ID NO: 7417	0.44 %	25
19	4192	SEQ ID NO: 7418	0.44 %	25
20	378	SEQ ID NO: 7419	0.4 %	22.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1867	SEQ ID NO: 7420	8 %	450
2	1495	SEQ ID NO: 7421	4 %	225
3	3921	SEQ ID NO: 7422	2.4 %	135
4	486	SEQ ID NO: 7423	2.22 %	125
5	4139	SEQ ID NO: 7424	2.22 %	125

6	62	SEQ ID NO: 7425	1.6 %	90
7	1190	SEQ ID NO: 7426	1.6 %	90
8	1284	SEQ ID NO: 7427	1.6 %	90
9	3284	SEQ ID NO: 7428	1.6 %	90
10	2921	SEQ ID NO: 7429	1.2 %	67.5
11	349	SEQ ID NO: 7430	0.8 %	45
12	789	SEQ ID NO: 7431	0.8 %	45
13	1185	SEQ ID NO: 7432	0.8 %	45
14	4184	SEQ ID NO: 7433	0.8 %	45
15	1313	SEQ ID NO: 7434	0.64 %	36
16	3948	SEQ ID NO: 7435	0.48 %	27
17	149	SEQ ID NO: 7436	0.44 %	25
18	941	SEQ ID NO: 7437	0.44 %	25
19	1390	SEQ ID NO: 7438	0.44 %	25
20	1613	SEQ ID NO: 7439	0.44 %	25

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1010	SEQ ID NO: 7440	1.48 %	180
2	3155	SEQ ID NO: 7441	1.48 %	180
3	1229	SEQ ID NO: 7442	1.23 %	150
4	2405	SEQ ID NO: 7443	0.88 %	108
5	2	SEQ ID NO: 7444	0.74 %	90
6	2304	SEQ ID NO: 7445	0.74 %	90
7	2358	SEQ ID NO: 7446	0.74 %	90
8	3160	SEQ ID NO: 7447	0.74 %	90
9	3771	SEQ ID NO: 7448	0.74 %	90
10	4007	SEQ ID NO: 7449	0.74 %	90
11	3079	SEQ ID NO: 7450	0.66 %	81
12	4045	SEQ ID NO: 7451	0.66 %	81
13	1081	SEQ ID NO: 7452	0.49 %	60
14	3268	SEQ ID NO: 7453	0.49 %	60
15	4144	SEQ ID NO: 7454	0.49 %	60
16	614	SEQ ID NO: 7455	0.37 %	45
17	728	SEQ ID NO: 7456	0.37 %	45
18	1537	SEQ ID NO: 7457	0.37 %	45
19	313	SEQ ID NO: 7458	0.32 %	40
20	1744	SEQ ID NO: 7459	0.32 %	40

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	62	SEQ ID NO: 7460	4.44 %	540
2	2151	SEQ ID NO: 7461	2.46 %	300
3	633	SEQ ID NO: 7462	2.22 %	270
4	1158	SEQ ID NO: 7463	2.22 %	270
5	2565	SEQ ID NO: 7464	2.22 %	270
6	2298	SEQ ID NO: 7465	1.77 %	216
7	3159	SEQ ID NO: 7466	1.11 %	135
8	640	SEQ ID NO: 7467	0.98 %	120
9	2186	SEQ ID NO: 7468	0.74 %	90
10	3869	SEQ ID NO: 7469	0.74 %	90
11	2308	SEQ ID NO: 7470	0.66 %	81
12	786	SEQ ID NO: 7471	0.55 %	67.5
13	749	SEQ ID NO: 7472	0.49 %	60
14	1080	SEQ ID NO: 7473	0.49 %	60
15	2358	SEQ ID NO: 7474	0.49 %	60
16	3955	SEQ ID NO: 7475	0.49 %	60
17	714	SEQ ID NO: 7476	0.37 %	45
18	1081	SEQ ID NO: 7477	0.37 %	45
19	1170	SEQ ID NO: 7478	0.37 %	45
20	1228	SEQ ID NO: 7479	0.37 %	45

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	3797	SEQ ID NO: 7480	37.57 %	600
2	4202	SEQ ID NO: 7481	37.57 %	600
3	3189	SEQ ID NO: 7482	25.05 %	400
4	1864	SEQ ID NO: 7483	23.14 %	369.6
5	1066	SEQ ID NO: 7484	22.54 %	360
6	2143	SEQ ID NO: 7485	22.54 %	360
7	2693	SEQ ID NO: 7486	22.54 %	360
8	1426	SEQ ID NO: 7487	18.78 %	300
9	1238	SEQ ID NO: 7488	18.03 %	288
10	3768	SEQ ID NO: 7489	18.03 %	288
11	797	SEQ ID NO: 7490	15.03 %	240

12	1882	SEQ ID NO: 7491	15.03 %	240
13	1490	SEQ ID NO: 7492	13.77 %	220
14	2237	SEQ ID NO: 7493	13.77 %	220
15	95	SEQ ID NO: 7494	12.52 %	200
16	1821	SEQ ID NO: 7495	12.52 %	200
17	2289	SEQ ID NO: 7496	12.52 %	200
18	3080	SEQ ID NO: 7497	12.52 %	200
19	3660	SEQ ID NO: 7498	12.52 %	200
20	4354	SEQ ID NO: 7499	12.52 %	200

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	2143	SEQ ID NO: 7500	37.87 %	604.8
2	1159	SEQ ID NO: 7501	26.30 %	420
3	1650	SEQ ID NO: 7502	26.30 %	420
4	1150	SEQ ID NO: 7503	18.78 %	300
5	2763	SEQ ID NO: 7504	18.78 %	300
6	3165	SEQ ID NO: 7505	18.78 %	300
7	3201	SEQ ID NO: 7506	15.03 %	240
8	3694	SEQ ID NO: 7507	15.03 %	240
9	4204	SEQ ID NO: 7508	15.03 %	240
10	1692	SEQ ID NO: 7509	13.77 %	220
11	797	SEQ ID NO: 7510	12.52 %	200
12	1610	SEQ ID NO: 7511	12.52 %	200
13	1789	SEQ ID NO: 7512	12.52 %	200
14	1881	SEQ ID NO: 7513	12.52 %	200
15	3090	SEQ ID NO: 7514	12.52 %	200
16	3763	SEQ ID NO: 7515	12.52 %	200
17	2569	SEQ ID NO: 7516	11.27 %	180
18	194	SEQ ID NO: 7517	9.39 %	150
19	1771	SEQ ID NO: 7518	9.39 %	150
20	2488	SEQ ID NO: 7519	9.39 %	150

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	2308	SEQ ID NO: 7520	0.20 %	8144.13515256
2	3729	SEQ ID NO: 7521	0.10 %	4047.23088

3	3574	SEQ ID NO: 7522	0.09 %	3547.4996634
4	3615	SEQ ID NO: 7523	0.06 %	2722.682592
5	3159	SEQ ID NO: 7524	0.05 %	1999.734264
6	2339	SEQ ID NO: 7525	0.03 %	1551.92907744
7	2201	SEQ ID NO: 7526	0.03 %	1521.53694
8	3559	SEQ ID NO: 7527	0.02 %	1174.38939504
9	3085	SEQ ID NO: 7528	0.02 %	1146.296448
10	4070	SEQ ID NO: 7529	0.02 %	970.4103696
11	3708	SEQ ID NO: 7530	0.02 %	958.92888
12	3098	SEQ ID NO: 7531	0.02 %	942.678
13	1362	SEQ ID NO: 7532	0.02 %	900.6984
14	3563	SEQ ID NO: 7533	0.01 %	735.86016
15	3774	SEQ ID NO: 7534	0.01 %	687.655656
16	4242	SEQ ID NO: 7535	0.01 %	685.78272
17	2340	SEQ ID NO: 7536	0.01 %	668.37342936
18	650	SEQ ID NO: 7537	0.01 %	640.1983392
19	3862	SEQ ID NO: 7538	0.01 %	620.57772
20	2860	SEQ ID NO: 7539	0.01 %	607.88448

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	2307	SEQ ID NO: 7540	0.40 %	15915.66281448
2	2201	SEQ ID NO: 7541	0.12 %	4772.09313
3	3558	SEQ ID NO: 7542	0.05 %	2295.04855632
4	1772	SEQ ID NO: 7543	0.04 %	1759.6656
5	3087	SEQ ID NO: 7544	0.03 %	1215.76896
6	2339	SEQ ID NO: 7545	0.02 %	1116.29986272
7	2308	SEQ ID NO: 7546	0.02 %	970.14776112
8	3061	SEQ ID NO: 7547	0.02 %	836.2525104
9	2748	SEQ ID NO: 7548	0.01 %	726.706344
10	3837	SEQ ID NO: 7549	0.01 %	720.8292
11	59	SEQ ID NO: 7550	0.01 %	650.3112
12	2877	SEQ ID NO: 7551	0.01 %	620.22996
13	4114	SEQ ID NO: 7552	0.01 %	559.8936
14	805	SEQ ID NO: 7553	0.01 %	484.4565072
15	1655	SEQ ID NO: 7554	0.01 %	437.48208
16	611	SEQ ID NO: 7555	0.00 %	319.9392
17	1961	SEQ ID NO: 7556	0.00 %	305.94186

18	1223	SEQ ID NO: 7557	0.00 %	289.08792
19	852	SEQ ID NO: 7558	0.00 %	285.67242
20	2139	SEQ ID NO: 7559	0.00 %	284.845869

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	Start position	Sequence	% of max. score	Score
1	4200	SEQ ID NO: 7560	50 %	18
2	281	SEQ ID NO: 7561	25 %	9
3	3236	SEQ ID NO: 7562	25 %	9
4	509	SEQ ID NO: 7563	16.66 %	6
5	848	SEQ ID NO: 7564	16.66 %	6
6	2193	SEQ ID NO: 7565	16.66 %	6
7	3542	SEQ ID NO: 7566	16.66 %	6
8	541	SEQ ID NO: 7567	15 %	5.4
9	1748	SEQ ID NO: 7568	12.5 %	4.5
10	829	SEQ ID NO: 7569	11.11 %	4
11	1149	SEQ ID NO: 7570	11.11 %	4
12	2027	SEQ ID NO: 7571	11.11 %	4
13	2576	SEQ ID NO: 7572	11.11 %	4
14	873	SEQ ID NO: 7573	8.33 %	3
15	2725	SEQ ID NO: 7574	8.33 %	3
16	3541	SEQ ID NO: 7575	8.33 %	3
17	1837	SEQ ID NO: 7576	7.5 %	2.7
18	2475	SEQ ID NO: 7577	7.5 %	2.7
19	2703	SEQ ID NO: 7578	7.5 %	2.7
20	1823	SEQ ID NO: 7579	6.66 %	2.4

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	Start position	Sequence	% of max. score	Score
1	3541	SEQ ID NO: 7580	50 %	18
2	281	SEQ ID NO: 7581	25 %	9
3	1495	SEQ ID NO: 7582	25 %	9
4	2303	SEQ ID NO: 7583	25 %	9
5	2616	SEQ ID NO: 7584	25 %	9
6	48	SEQ ID NO: 7585	16.66 %	6
7	1394	SEQ ID NO: 7586	16.66 %	6
8	1499	SEQ ID NO: 7587	16.66 %	6

9	1862	SEQ ID NO: 7588	16.66 %	6
10	1163	SEQ ID NO: 7589	11.11 %	4
11	4006	SEQ ID NO: 7590	11.11 %	4
12	4344	SEQ ID NO: 7591	11.11 %	4
13	633	SEQ ID NO: 7592	10 %	3.6
14	119	SEQ ID NO: 7593	8.33 %	3
15	1190	SEQ ID NO: 7594	8.33 %	3
16	1195	SEQ ID NO: 7595	8.33 %	3
17	1725	SEQ ID NO: 7596	8.33 %	3
18	2728	SEQ ID NO: 7597	8.33 %	3
19	2895	SEQ ID NO: 7598	8.33 %	3
20	3033	SEQ ID NO: 7599	8.33 %	3

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1335	SEQ ID NO: 7600	4.44 %	240
2	2580	SEQ ID NO: 7601	4.44 %	240
3	1703	SEQ ID NO: 7602	3.70 %	200
4	113	SEQ ID NO: 7603	2.22 %	120
5	168	SEQ ID NO: 7604	2.22 %	120
6	2842	SEQ ID NO: 7605	2.22 %	120
7	4027	SEQ ID NO: 7606	2.22 %	120
8	3680	SEQ ID NO: 7607	1.66 %	90
9	2085	SEQ ID NO: 7608	1.48 %	80
10	2492	SEQ ID NO: 7609	1.48 %	80
11	2660	SEQ ID NO: 7610	1.48 %	80
12	2906	SEQ ID NO: 7611	1.48 %	80
13	3346	SEQ ID NO: 7612	1.48 %	80
14	4038	SEQ ID NO: 7613	1.48 %	80
15	1163	SEQ ID NO: 7614	1.11 %	60
16	1457	SEQ ID NO: 7615	1.11 %	60
17	2351	SEQ ID NO: 7616	1.11 %	60
18	2471	SEQ ID NO: 7617	1.11 %	60
19	3499	SEQ ID NO: 7618	1.11 %	60
20	3635	SEQ ID NO: 7619	1.11 %	60

HLA B7 - 10 mers	
Maximum possible score using this molecule type	5400

Rank	Start position	Sequence	% of max. score	Score
1	1703	SEQ ID NO: 7620	3.70 %	200
2	17	SEQ ID NO: 7621	2.22 %	120
3	3008	SEQ ID NO: 7622	2.22 %	120
4	4106	SEQ ID NO: 7623	2.22 %	120
5	3450	SEQ ID NO: 7624	1.66 %	90
6	113	SEQ ID NO: 7625	1.48 %	80
7	195	SEQ ID NO: 7626	1.48 %	80
8	307	SEQ ID NO: 7627	1.48 %	80
9	780	SEQ ID NO: 7628	1.48 %	80
10	1000	SEQ ID NO: 7629	1.48 %	80
11	1072	SEQ ID NO: 7630	1.48 %	80
12	1404	SEQ ID NO: 7631	1.48 %	80
13	1980	SEQ ID NO: 7632	1.48 %	80
14	2262	SEQ ID NO: 7633	1.48 %	80
15	2543	SEQ ID NO: 7634	1.48 %	80
16	2906	SEQ ID NO: 7635	1.48 %	80
17	3077	SEQ ID NO: 7636	1.48 %	80
18	3175	SEQ ID NO: 7637	1.48 %	80
19	4195	SEQ ID NO: 7638	1.48 %	80
20	4251	SEQ ID NO: 7639	1.48 %	80

Table 14: Epitopes for SEQ ID NO: 6040

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	20	SEQ ID NO: 7640	0.04 %	2.25
2	91	SEQ ID NO: 7641	0.01 %	1
3	125	SEQ ID NO: 7642	0.01 %	0.75
4	56	SEQ ID NO: 7643	0.00 %	0.5
5	145	SEQ ID NO: 7644	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	20	SEQ ID NO: 7645	0.01 %	0.9
2	56	SEQ ID NO: 7646	0.00 %	0.5
3	71	SEQ ID NO: 7647	0.00 %	0.5

4	144	SEQ ID NO: 7648	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	Start position	Sequence	% of max. score	Score
1	115	SEQ ID NO: 7649	0.24 %	30
2	87	SEQ ID NO: 7650	0.04 %	6
3	80	SEQ ID NO: 7651	0.03 %	4.05
4	125	SEQ ID NO: 7652	0.01 %	1.8
5	39	SEQ ID NO: 7653	0.01 %	1.5
6	56	SEQ ID NO: 7654	0.01 %	1.5
7	135	SEQ ID NO: 7655	0.00 %	1.2
8	91	SEQ ID NO: 7656	0.00 %	1
9	119	SEQ ID NO: 7657	0.00 %	1
10	141	SEQ ID NO: 7658	0.00 %	0.9
11	150	SEQ ID NO: 7659	0.00 %	0.6
12	137	SEQ ID NO: 7660	0.00 %	0.54

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	Start position	Sequence	% of max. score	Score
1	36	SEQ ID NO: 7661	0.24 %	30
2	144	SEQ ID NO: 7662	0.06 %	8
3	101	SEQ ID NO: 7663	0.03 %	4
4	99	SEQ ID NO: 7664	0.02 %	3.6
5	80	SEQ ID NO: 7665	0.02 %	2.7
6	125	SEQ ID NO: 7666	0.01 %	1.6875
7	71	SEQ ID NO: 7667	0.01 %	1.5
8	118	SEQ ID NO: 7668	0.01 %	1.5
9	40	SEQ ID NO: 7669	0.01 %	1.35
10	5	SEQ ID NO: 7670	0.00 %	0.9
11	56	SEQ ID NO: 7671	0.00 %	0.9
12	107	SEQ ID NO: 7672	0.00 %	0.6
13	135	SEQ ID NO: 7673	0.00 %	0.6
14	141	SEQ ID NO: 7674	0.00 %	0.6
15	148	SEQ ID NO: 7675	0.00 %	0.6
16	116	SEQ ID NO: 7676	0.00 %	0.5

HLA A24 - 9 mers

Maximum possible score using this molecule type				1596.672
Rank	Start position	Sequence	% of max. score	Score
1	153	SEQ ID NO: 7677	1.05 %	16.8
2	80	SEQ ID NO: 7678	0.75 %	12
3	123	SEQ ID NO: 7679	0.50 %	8
4	137	SEQ ID NO: 7680	0.50 %	8
5	9	SEQ ID NO: 7681	0.45 %	7.2
6	77	SEQ ID NO: 7682	0.45 %	7.2
7	112	SEQ ID NO: 7683	0.45 %	7.2
8	73	SEQ ID NO: 7684	0.41 %	6.6
9	32	SEQ ID NO: 7685	0.37 %	6
10	110	SEQ ID NO: 7686	0.37 %	6
11	140	SEQ ID NO: 7687	0.37 %	6
12	143	SEQ ID NO: 7688	0.37 %	6
13	18	SEQ ID NO: 7689	0.30 %	4.8
14	54	SEQ ID NO: 7690	0.30 %	4.8
15	108	SEQ ID NO: 7691	0.30 %	4.8
16	141	SEQ ID NO: 7692	0.30 %	4.8
17	92	SEQ ID NO: 7693	0.27 %	4.4
18	33	SEQ ID NO: 7694	0.25 %	4
19	49	SEQ ID NO: 7695	0.25 %	4
20	111	SEQ ID NO: 7696	0.25 %	4

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	Start position	Sequence	% of max. score	Score
1	142	SEQ ID NO: 7697	12.52 %	200
2	110	SEQ ID NO: 7698	0.75 %	12
3	99	SEQ ID NO: 7699	0.50 %	8
4	8	SEQ ID NO: 7700	0.45 %	7.2
5	140	SEQ ID NO: 7701	0.45 %	7.2
6	32	SEQ ID NO: 7702	0.37 %	6
7	17	SEQ ID NO: 7703	0.30 %	4.8
8	53	SEQ ID NO: 7704	0.30 %	4.8
9	76	SEQ ID NO: 7705	0.30 %	4.8
10	107	SEQ ID NO: 7706	0.30 %	4.8
11	111	SEQ ID NO: 7707	0.30 %	4.8
12	72	SEQ ID NO: 7708	0.27 %	4.4
13	91	SEQ ID NO: 7709	0.27 %	4.4

14	31	SEQ ID NO: 7710	0.25 %	4
15	127	SEQ ID NO: 7711	0.25 %	4
16	139	SEQ ID NO: 7712	0.25 %	4
17	80	SEQ ID NO: 7713	0.22 %	3.6
18	38	SEQ ID NO: 7714	0.18 %	3
19	118	SEQ ID NO: 7715	0.18 %	3
20	49	SEQ ID NO: 7716	0.12 %	2

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	80	SEQ ID NO: 7717	0.00 %	171.96732
2	147	SEQ ID NO: 7718	0.00 %	51.46848
3	143	SEQ ID NO: 7719	0.00 %	11.6146182
4	56	SEQ ID NO: 7720	0.00 %	11.304684
5	10	SEQ ID NO: 7721	0.00 %	10.34586
6	6	SEQ ID NO: 7722	0.00 %	6.56830734
7	26	SEQ ID NO: 7723	0.00 %	6.07614
8	141	SEQ ID NO: 7724	0.00 %	5.981472
9	148	SEQ ID NO: 7725	0.00 %	5.194044
10	9	SEQ ID NO: 7726	0.00 %	4.299183
11	137	SEQ ID NO: 7727	0.00 %	4.299183
12	130	SEQ ID NO: 7728	0.00 %	4.138344
13	84	SEQ ID NO: 7729	0.00 %	3.42792
14	27	SEQ ID NO: 7730	0.00 %	3.383484
15	2	SEQ ID NO: 7731	0.00 %	3.381
16	62	SEQ ID NO: 7732	0.00 %	3.251556
17	23	SEQ ID NO: 7733	0.00 %	2.9542005
18	99	SEQ ID NO: 7734	0.00 %	1.982232
19	33	SEQ ID NO: 7735	0.00 %	1.86921
20	111	SEQ ID NO: 7736	0.00 %	1.76402985

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	5	SEQ ID NO: 7737	0.00 %	159.9696
2	25	SEQ ID NO: 7738	0.00 %	69.552
3	80	SEQ ID NO: 7739	0.00 %	36.5148
4	107	SEQ ID NO: 7740	0.00 %	21.3624

5	148	SEQ ID NO: 7741	0.00 %	17.73576
6	61	SEQ ID NO: 7742	0.00 %	13.9104
7	147	SEQ ID NO: 7743	0.00 %	11.304684
8	53	SEQ ID NO: 7744	0.00 %	8.230458
9	17	SEQ ID NO: 7745	0.00 %	7.3086111
10	110	SEQ ID NO: 7746	0.00 %	6.174104475
11	9	SEQ ID NO: 7747	0.00 %	6.0858
12	99	SEQ ID NO: 7748	0.00 %	5.6823984
13	2	SEQ ID NO: 7749	0.00 %	3.188283
14	41	SEQ ID NO: 7750	0.00 %	2.206413
15	135	SEQ ID NO: 7751	0.00 %	2.076624
16	76	SEQ ID NO: 7752	0.00 %	2.005692
17	23	SEQ ID NO: 7753	0.00 %	1.798209
18	40	SEQ ID NO: 7754	0.00 %	1.68996456
19	39	SEQ ID NO: 7755	0.00 %	1.516482
20	118	SEQ ID NO: 7756	0.00 %	1.2683304

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	91	SEQ ID NO: 7757	2.77 %	1

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	101	SEQ ID NO: 7758	33.33 %	12
2	71	SEQ ID NO: 7759	2.77 %	1
3	90	SEQ ID NO: 7760	1.66 %	0.6

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	49	SEQ ID NO: 7761	2.22 %	120
2	9	SEQ ID NO: 7762	1.11 %	60
3	73	SEQ ID NO: 7763	0.66 %	36
4	33	SEQ ID NO: 7764	0.37 %	20
5	137	SEQ ID NO: 7765	0.37 %	20
6	141	SEQ ID NO: 7766	0.37 %	20
7	77	SEQ ID NO: 7767	0.22 %	12

8	112	SEQ ID NO: 7768	0.22 %	12
9	143	SEQ ID NO: 7769	0.22 %	12
10	81	SEQ ID NO: 7770	0.14 %	8
11	13	SEQ ID NO: 7771	0.09 %	5
12	69	SEQ ID NO: 7772	0.09 %	5
13	18	SEQ ID NO: 7773	0.07 %	4
14	32	SEQ ID NO: 7774	0.07 %	4
15	54	SEQ ID NO: 7775	0.07 %	4
16	80	SEQ ID NO: 7776	0.07 %	4
17	92	SEQ ID NO: 7777	0.07 %	4
18	108	SEQ ID NO: 7778	0.07 %	4
19	111	SEQ ID NO: 7779	0.07 %	4
20	123	SEQ ID NO: 7780	0.07 %	4

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	99	SEQ ID NO: 7781	0.74 %	40
2	17	SEQ ID NO: 7782	0.37 %	20
3	8	SEQ ID NO: 7783	0.22 %	12
4	72	SEQ ID NO: 7784	0.22 %	12
5	91	SEQ ID NO: 7785	0.22 %	12
6	127	SEQ ID NO: 7786	0.11 %	6
7	31	SEQ ID NO: 7787	0.07 %	4
8	32	SEQ ID NO: 7788	0.07 %	4
9	53	SEQ ID NO: 7789	0.07 %	4
10	76	SEQ ID NO: 7790	0.07 %	4
11	107	SEQ ID NO: 7791	0.07 %	4
12	110	SEQ ID NO: 7792	0.07 %	4
13	111	SEQ ID NO: 7793	0.07 %	4
14	140	SEQ ID NO: 7794	0.07 %	4
15	9	SEQ ID NO: 7795	0.05 %	3
16	19	SEQ ID NO: 7796	0.05 %	3
17	33	SEQ ID NO: 7797	0.03 %	2
18	93	SEQ ID NO: 7798	0.03 %	2
19	102	SEQ ID NO: 7799	0.03 %	2
20	129	SEQ ID NO: 7800	0.02 %	1.5

Table 15: Epitopes for SEQ ID NO: 6041

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1818	SEQ ID NO: 7801	1.6 %	90
2	373	SEQ ID NO: 7802	1.33 %	75
3	681	SEQ ID NO: 7803	1.33 %	75
4	74	SEQ ID NO: 7804	0.88 %	50
5	786	SEQ ID NO: 7805	0.88 %	50
6	1495	SEQ ID NO: 7806	0.88 %	50
7	88	SEQ ID NO: 7807	0.8 %	45
8	357	SEQ ID NO: 7808	0.8 %	45
9	1271	SEQ ID NO: 7809	0.8 %	45
10	1799	SEQ ID NO: 7810	0.8 %	45
11	1393	SEQ ID NO: 7811	0.48 %	27
12	386	SEQ ID NO: 7812	0.44 %	25
13	2304	SEQ ID NO: 7813	0.44 %	25
14	198	SEQ ID NO: 7814	0.4 %	22.5
15	840	SEQ ID NO: 7815	0.4 %	22.5
16	2359	SEQ ID NO: 7816	0.4 %	22.5
17	1194	SEQ ID NO: 7817	0.32 %	18
18	1546	SEQ ID NO: 7818	0.32 %	18
19	2200	SEQ ID NO: 7819	0.22 %	12.5
20	996	SEQ ID NO: 7820	0.2 %	11.25

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	995	SEQ ID NO: 7821	10 %	562.5
2	1303	SEQ ID NO: 7822	2.22 %	125
3	1582	SEQ ID NO: 7823	2 %	112.5
4	1456	SEQ ID NO: 7824	1.6 %	90
5	772	SEQ ID NO: 7825	1.11 %	62.5
6	181	SEQ ID NO: 7826	0.88 %	50
7	632	SEQ ID NO: 7827	0.88 %	50
8	2281	SEQ ID NO: 7828	0.88 %	50
9	1586	SEQ ID NO: 7829	0.8 %	45
10	2109	SEQ ID NO: 7830	0.8 %	45
11	745	SEQ ID NO: 7831	0.55 %	31.25

12	1916	SEQ ID NO: 7832	0.53 %	30
13	966	SEQ ID NO: 7833	0.44 %	25
14	1387	SEQ ID NO: 7834	0.44 %	25
15	2263	SEQ ID NO: 7835	0.44 %	25
16	2457	SEQ ID NO: 7836	0.26 %	15
17	1057	SEQ ID NO: 7837	0.22 %	12.5
18	2562	SEQ ID NO: 7838	0.22 %	12.5
19	74	SEQ ID NO: 7839	0.17 %	10
20	298	SEQ ID NO: 7840	0.17 %	10

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	536	SEQ ID NO: 7841	3.33 %	405
2	986	SEQ ID NO: 7842	2.46 %	300
3	805	SEQ ID NO: 7843	1.64 %	200
4	2345	SEQ ID NO: 7844	1.48 %	180
5	2481	SEQ ID NO: 7845	0.55 %	67.5
6	204	SEQ ID NO: 7846	0.49 %	60
7	895	SEQ ID NO: 7847	0.44 %	54
8	1512	SEQ ID NO: 7848	0.44 %	54
9	2491	SEQ ID NO: 7849	0.37 %	45
10	436	SEQ ID NO: 7850	0.32 %	40
11	917	SEQ ID NO: 7851	0.32 %	40
12	1176	SEQ ID NO: 7852	0.32 %	40
13	1517	SEQ ID NO: 7853	0.29 %	36
14	466	SEQ ID NO: 7854	0.24 %	30
15	1784	SEQ ID NO: 7855	0.24 %	30
16	2039	SEQ ID NO: 7856	0.24 %	30
17	2124	SEQ ID NO: 7857	0.24 %	30
18	1049	SEQ ID NO: 7858	0.22 %	27
19	2200	SEQ ID NO: 7859	0.22 %	27
20	2598	SEQ ID NO: 7860	0.22 %	27

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	392	SEQ ID NO: 7861	2.46 %	300
2	2230	SEQ ID NO: 7862	1.48 %	180

3	590	SEQ ID NO: 7863	1.11 %	135
4	697	SEQ ID NO: 7864	1.11 %	135
5	919	SEQ ID NO: 7865	0.74 %	90
6	1354	SEQ ID NO: 7866	0.74 %	90
7	1430	SEQ ID NO: 7867	0.74 %	90
8	2534	SEQ ID NO: 7868	0.74 %	90
9	202	SEQ ID NO: 7869	0.49 %	60
10	488	SEQ ID NO: 7870	0.49 %	60
11	922	SEQ ID NO: 7871	0.49 %	60
12	1735	SEQ ID NO: 7872	0.49 %	60
13	2281	SEQ ID NO: 7873	0.49 %	60
14	1894	SEQ ID NO: 7874	0.44 %	54
15	2552	SEQ ID NO: 7875	0.44 %	54
16	555	SEQ ID NO: 7876	0.37 %	45
17	1134	SEQ ID NO: 7877	0.37 %	45
18	1149	SEQ ID NO: 7878	0.29 %	36
19	283	SEQ ID NO: 7879	0.24 %	30
20	917	SEQ ID NO: 7880	0.24 %	30

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	2375	SEQ ID NO: 7881	36.07 %	576
2	1751	SEQ ID NO: 7882	28.93 %	462
3	195	SEQ ID NO: 7883	25.05 %	400
4	2306	SEQ ID NO: 7884	21.04 %	336
5	806	SEQ ID NO: 7885	20.66 %	330
6	1252	SEQ ID NO: 7886	18.78 %	300
7	160	SEQ ID NO: 7887	15.03 %	240
8	517	SEQ ID NO: 7888	15.03 %	240
9	375	SEQ ID NO: 7889	12.52 %	200
10	1275	SEQ ID NO: 7890	12.52 %	200
11	2175	SEQ ID NO: 7891	12.52 %	200
12	2207	SEQ ID NO: 7892	12.52 %	200
13	2343	SEQ ID NO: 7893	12.52 %	200
14	443	SEQ ID NO: 7894	11.27 %	180
15	668	SEQ ID NO: 7895	7.51 %	120
16	1825	SEQ ID NO: 7896	6.88 %	110
17	1690	SEQ ID NO: 7897	4.69 %	75

18	159	SEQ ID NO: 7898	3.75 %	60
19	2550	SEQ ID NO: 7899	3.75 %	60
20	1949	SEQ ID NO: 7900	3.38 %	54

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	641	SEQ ID NO: 7901	45.09 %	720
2	809	SEQ ID NO: 7902	24.80 %	396
3	1209	SEQ ID NO: 7903	22.54 %	360
4	216	SEQ ID NO: 7904	18.03 %	288
5	159	SEQ ID NO: 7905	15.03 %	240
6	528	SEQ ID NO: 7906	15.03 %	240
7	799	SEQ ID NO: 7907	15.03 %	240
8	1436	SEQ ID NO: 7908	15.03 %	240
9	2219	SEQ ID NO: 7909	15.03 %	240
10	1065	SEQ ID NO: 7910	13.77 %	220
11	1953	SEQ ID NO: 7911	13.15 %	210
12	1966	SEQ ID NO: 7912	12.52 %	200
13	2600	SEQ ID NO: 7913	12.52 %	200
14	71	SEQ ID NO: 7914	9.39 %	150
15	380	SEQ ID NO: 7915	9.39 %	150
16	1989	SEQ ID NO: 7916	9.39 %	150
17	342	SEQ ID NO: 7917	8.76 %	140
18	1071	SEQ ID NO: 7918	8.76 %	140
19	2570	SEQ ID NO: 7919	6.88 %	110
20	2550	SEQ ID NO: 7920	6.26 %	100

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1632	SEQ ID NO: 7921	0.09 %	3607.31448
2	1640	SEQ ID NO: 7922	0.04 %	1748.2560912
3	1776	SEQ ID NO: 7923	0.03 %	1492.58592
4	2512	SEQ ID NO: 7924	0.03 %	1434.16845
5	1073	SEQ ID NO: 7925	0.03 %	1338.876
6	230	SEQ ID NO: 7926	0.01 %	685.78272
7	1001	SEQ ID NO: 7927	0.01 %	559.8936
8	716	SEQ ID NO: 7928	0.01 %	558.27486

9	2280	SEQ ID NO: 7929	0.01 %	511.19781048
10	590	SEQ ID NO: 7930	0.01 %	469.6692
11	664	SEQ ID NO: 7931	0.01 %	442.076389524
12	1094	SEQ ID NO: 7932	0.00 %	382.536
13	1735	SEQ ID NO: 7933	0.00 %	382.536
14	1625	SEQ ID NO: 7934	0.00 %	342.4606344
15	1974	SEQ ID NO: 7935	0.00 %	336.885048
16	2382	SEQ ID NO: 7936	0.00 %	319.9392
17	2417	SEQ ID NO: 7937	0.00 %	319.9392
18	744	SEQ ID NO: 7938	0.00 %	256.416670125
19	108	SEQ ID NO: 7939	0.00 %	232.52724
20	390	SEQ ID NO: 7940	0.00 %	228.0411084

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	2511	SEQ ID NO: 7941	0.38 %	15126.90795
2	1608	SEQ ID NO: 7942	0.05 %	2049.4656
3	2572	SEQ ID NO: 7943	0.04 %	1879.5921264
4	255	SEQ ID NO: 7944	0.03 %	1566.6522795
5	895	SEQ ID NO: 7945	0.03 %	1338.876
6	1171	SEQ ID NO: 7946	0.02 %	1107.960876
7	1691	SEQ ID NO: 7947	0.01 %	782.95521024
8	20	SEQ ID NO: 7948	0.01 %	549.9372312
9	1632	SEQ ID NO: 7949	0.01 %	479.041993296
10	2280	SEQ ID NO: 7950	0.01 %	472.418344576987
11	1963	SEQ ID NO: 7951	0.00 %	358.73928
12	1955	SEQ ID NO: 7952	0.00 %	331.093464
13	741	SEQ ID NO: 7953	0.00 %	318.652488
14	523	SEQ ID NO: 7954	0.00 %	278.7876
15	1073	SEQ ID NO: 7955	0.00 %	266.6988828
16	2489	SEQ ID NO: 7956	0.00 %	243.432
17	777	SEQ ID NO: 7957	0.00 %	218.5730664
18	1737	SEQ ID NO: 7958	0.00 %	218.0785572
19	589	SEQ ID NO: 7959	0.00 %	210.538251
20	229	SEQ ID NO: 7960	0.00 %	205.230564

HLA A 1101 - 9 mers	
Maximum possible score using this molecule type	36

Rank	Start position	Sequence	% of max. score	Score
1	2337	SEQ ID NO: 7961	33.33 %	12
2	2156	SEQ ID NO: 7962	25 %	9
3	492	SEQ ID NO: 7963	20 %	7.2
4	18	SEQ ID NO: 7964	16.66 %	6
5	332	SEQ ID NO: 7965	16.66 %	6
6	415	SEQ ID NO: 7966	16.66 %	6
7	2479	SEQ ID NO: 7967	16.66 %	6
8	1495	SEQ ID NO: 7968	11.11 %	4
9	2035	SEQ ID NO: 7969	11.11 %	4
10	1349	SEQ ID NO: 7970	10 %	3.6
11	1194	SEQ ID NO: 7971	8.33 %	3
12	1648	SEQ ID NO: 7972	8.33 %	3
13	96	SEQ ID NO: 7973	6.66 %	2.4
14	764	SEQ ID NO: 7974	6.66 %	2.4
15	986	SEQ ID NO: 7975	6.66 %	2.4
16	2345	SEQ ID NO: 7976	6.66 %	2.4
17	698	SEQ ID NO: 7977	5.55 %	2
18	1355	SEQ ID NO: 7978	5.55 %	2
19	1987	SEQ ID NO: 7979	5.55 %	2
20	2085	SEQ ID NO: 7980	5.55 %	2

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	Start position	Sequence	% of max. score	Score
1	2083	SEQ ID NO: 7981	33.33 %	12
2	2123	SEQ ID NO: 7982	25 %	9
3	2147	SEQ ID NO: 7983	16.66 %	6
4	331	SEQ ID NO: 7984	12.5 %	4.5
5	1035	SEQ ID NO: 7985	11.11 %	4
6	1064	SEQ ID NO: 7986	11.11 %	4
7	2154	SEQ ID NO: 7987	11.11 %	4
8	1048	SEQ ID NO: 7988	7.5 %	2.7
9	202	SEQ ID NO: 7989	6.66 %	2.4
10	721	SEQ ID NO: 7990	6.66 %	2.4
11	2109	SEQ ID NO: 7991	6.66 %	2.4
12	2230	SEQ ID NO: 7992	6.66 %	2.4
13	1306	SEQ ID NO: 7993	5.55 %	2
14	1622	SEQ ID NO: 7994	5.55 %	2

15	1772	SEQ ID NO: 7995	5.55 %	2
16	1796	SEQ ID NO: 7996	5.55 %	2
17	186	SEQ ID NO: 7997	5 %	1.8
18	414	SEQ ID NO: 7998	5 %	1.8
19	697	SEQ ID NO: 7999	5 %	1.8
20	1175	SEQ ID NO: 8000	5 %	1.8

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1447	SEQ ID NO: 8001	14.81 %	800
2	642	SEQ ID NO: 8002	3.70 %	200
3	34	SEQ ID NO: 8003	2.22 %	120
4	186	SEQ ID NO: 8004	1.48 %	80
5	244	SEQ ID NO: 8005	1.48 %	80
6	459	SEQ ID NO: 8006	1.48 %	80
7	1475	SEQ ID NO: 8007	1.48 %	80
8	1867	SEQ ID NO: 8008	1.48 %	80
9	2032	SEQ ID NO: 8009	1.48 %	80
10	2047	SEQ ID NO: 8010	1.48 %	80
11	2335	SEQ ID NO: 8011	1.48 %	80
12	622	SEQ ID NO: 8012	1.11 %	60
13	1375	SEQ ID NO: 8013	1.11 %	60
14	1617	SEQ ID NO: 8014	0.92 %	50
15	1023	SEQ ID NO: 8015	0.83 %	45
16	286	SEQ ID NO: 8016	0.74 %	40
17	490	SEQ ID NO: 8017	0.74 %	40
18	810	SEQ ID NO: 8018	0.74 %	40
19	1420	SEQ ID NO: 8019	0.74 %	40
20	1854	SEQ ID NO: 8020	0.74 %	40

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1617	SEQ ID NO: 8021	3.70 %	200
2	752	SEQ ID NO: 8022	2.22 %	120
3	1552	SEQ ID NO: 8023	2.22 %	120
4	154	SEQ ID NO: 8024	1.48 %	80
5	165	SEQ ID NO: 8025	1.48 %	80

6	383	SEQ ID NO: 8026	1.48 %	80
7	1501	SEQ ID NO: 8027	1.48 %	80
8	2093	SEQ ID NO: 8028	1.48 %	80
9	2564	SEQ ID NO: 8029	1.48 %	80
10	622	SEQ ID NO: 8030	1.11 %	60
11	1086	SEQ ID NO: 8031	1.11 %	60
12	1262	SEQ ID NO: 8032	1.11 %	60
13	1556	SEQ ID NO: 8033	1.11 %	60
14	845	SEQ ID NO: 8034	1 %	54
15	286	SEQ ID NO: 8035	0.74 %	40
16	490	SEQ ID NO: 8036	0.74 %	40
17	552	SEQ ID NO: 8037	0.74 %	40
18	1858	SEQ ID NO: 8038	0.74 %	40
19	2107	SEQ ID NO: 8039	0.74 %	40
20	2582	SEQ ID NO: 8040	0.74 %	40

Table 16: Epitopes for SEQ ID NO: 6042

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	846	SEQ ID NO: 8041	2.22 %	125
2	798	SEQ ID NO: 8042	1.6 %	90
3	787	SEQ ID NO: 8043	0.88 %	50
4	1178	SEQ ID NO: 8044	0.88 %	50
5	637	SEQ ID NO: 8045	0.8 %	45
6	557	SEQ ID NO: 8046	0.44 %	25
7	1020	SEQ ID NO: 8047	0.44 %	25
8	282	SEQ ID NO: 8048	0.32 %	18
9	1241	SEQ ID NO: 8049	0.24 %	13.5
10	466	SEQ ID NO: 8050	0.22 %	12.5
11	727	SEQ ID NO: 8051	0.2 %	11.25
12	706	SEQ ID NO: 8052	0.17 %	10
13	324	SEQ ID NO: 8053	0.16 %	9
14	752	SEQ ID NO: 8054	0.16 %	9
15	54	SEQ ID NO: 8055	0.13 %	7.5
16	554	SEQ ID NO: 8056	0.13 %	7.5
17	590	SEQ ID NO: 8057	0.12 %	6.75

18	569	SEQ ID NO: 8058	0.08 %	5
19	613	SEQ ID NO: 8059	0.08 %	5
20	90	SEQ ID NO: 8060	0.08 %	4.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1241	SEQ ID NO: 8061	4.8 %	270
2	967	SEQ ID NO: 8062	0.8 %	45
3	1010	SEQ ID NO: 8063	0.48 %	27
4	426	SEQ ID NO: 8064	0.44 %	25
5	809	SEQ ID NO: 8065	0.44 %	25
6	1178	SEQ ID NO: 8066	0.44 %	25
7	787	SEQ ID NO: 8067	0.22 %	12.5
8	958	SEQ ID NO: 8068	0.22 %	12.5
9	727	SEQ ID NO: 8069	0.2 %	11.25
10	610	SEQ ID NO: 8070	0.17 %	10
11	12	SEQ ID NO: 8071	0.13 %	7.5
12	1181	SEQ ID NO: 8072	0.12 %	6.75
13	373	SEQ ID NO: 8073	0.11 %	6.25
14	602	SEQ ID NO: 8074	0.11 %	6.25
15	20	SEQ ID NO: 8075	0.04 %	2.5
16	32	SEQ ID NO: 8076	0.04 %	2.5
17	53	SEQ ID NO: 8077	0.04 %	2.5
18	400	SEQ ID NO: 8078	0.04 %	2.5
19	557	SEQ ID NO: 8079	0.04 %	2.5
20	667	SEQ ID NO: 8080	0.04 %	2.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	768	SEQ ID NO: 8081	0.82 %	100
2	808	SEQ ID NO: 8082	0.49 %	60
3	85	SEQ ID NO: 8083	0.24 %	30
4	663	SEQ ID NO: 8084	0.24 %	30
5	1245	SEQ ID NO: 8085	0.14 %	18
6	288	SEQ ID NO: 8086	0.09 %	12
7	50	SEQ ID NO: 8087	0.08 %	10
8	320	SEQ ID NO: 8088	0.07 %	9

9	402	SEQ ID NO: 8089	0.07 %	9
10	798	SEQ ID NO: 8090	0.07 %	9
11	902	SEQ ID NO: 8091	0.06 %	8.1
12	364	SEQ ID NO: 8092	0.05 %	6.75
13	297	SEQ ID NO: 8093	0.04 %	6
14	992	SEQ ID NO: 8094	0.04 %	6
15	38	SEQ ID NO: 8095	0.03 %	4.5
16	249	SEQ ID NO: 8096	0.03 %	4.5
17	706	SEQ ID NO: 8097	0.03 %	4.05
18	1204	SEQ ID NO: 8098	0.03 %	4.05
19	1178	SEQ ID NO: 8099	0.03 %	4
20	343	SEQ ID NO: 8100	0.02 %	3.6

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	255	SEQ ID NO: 8101	1.48 %	180
2	180	SEQ ID NO: 8102	0.55 %	67.5
3	768	SEQ ID NO: 8103	0.49 %	60
4	1177	SEQ ID NO: 8104	0.49 %	60
5	380	SEQ ID NO: 8105	0.24 %	30
6	100	SEQ ID NO: 8106	0.18 %	22.5
7	786	SEQ ID NO: 8107	0.16 %	20
8	1217	SEQ ID NO: 8108	0.16 %	20
9	207	SEQ ID NO: 8109	0.14 %	18
10	1183	SEQ ID NO: 8110	0.14 %	18
11	38	SEQ ID NO: 8111	0.09 %	12
12	52	SEQ ID NO: 8112	0.09 %	12
13	8	SEQ ID NO: 8113	0.06 %	8
14	679	SEQ ID NO: 8114	0.06 %	8
15	73	SEQ ID NO: 8115	0.05 %	6.75
16	1204	SEQ ID NO: 8116	0.05 %	6.075
17	50	SEQ ID NO: 8117	0.04 %	6
18	774	SEQ ID NO: 8118	0.04 %	6
19	845	SEQ ID NO: 8119	0.04 %	6
20	214	SEQ ID NO: 8120	0.04 %	5.4

HLA A24 - 9 mers	
Maximum possible score using this molecule type	1596.672

Rank	Start position	Sequence	% of max. score	Score
1	1118	SEQ ID NO: 8121	19.84 %	316.8
2	51	SEQ ID NO: 8122	18.78 %	300
3	161	SEQ ID NO: 8123	18.78 %	300
4	434	SEQ ID NO: 8124	18.78 %	300
5	365	SEQ ID NO: 8125	13.77 %	220
6	736	SEQ ID NO: 8126	12.52 %	200
7	620	SEQ ID NO: 8127	7.51 %	120
8	1068	SEQ ID NO: 8128	7.51 %	120
9	817	SEQ ID NO: 8129	3.75 %	60
10	336	SEQ ID NO: 8130	3.44 %	55
11	687	SEQ ID NO: 8131	3.13 %	50
12	254	SEQ ID NO: 8132	2.34 %	37.5
13	627	SEQ ID NO: 8133	1.87 %	30
14	950	SEQ ID NO: 8134	1.75 %	28
15	28	SEQ ID NO: 8135	1.56 %	25
16	408	SEQ ID NO: 8136	1.56 %	25
17	159	SEQ ID NO: 8137	1.31 %	21
18	1166	SEQ ID NO: 8138	1.26 %	20.16
19	45	SEQ ID NO: 8139	1.25 %	20
20	185	SEQ ID NO: 8140	1.25 %	20

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	438	SEQ ID NO: 8141	27.55 %	440
2	489	SEQ ID NO: 8142	22.54 %	360
3	254	SEQ ID NO: 8143	18.78 %	300
4	354	SEQ ID NO: 8144	11.27 %	180
5	406	SEQ ID NO: 8145	11.27 %	180
6	1047	SEQ ID NO: 8146	11.27 %	180
7	473	SEQ ID NO: 8147	7.51 %	120
8	350	SEQ ID NO: 8148	6.26 %	100
9	769	SEQ ID NO: 8149	6.26 %	100
10	193	SEQ ID NO: 8150	5.63 %	90
11	479	SEQ ID NO: 8151	3.13 %	50
12	0	SEQ ID NO: 8152	2.70 %	43.2
13	813	SEQ ID NO: 8153	1.87 %	30
14	739	SEQ ID NO: 8154	1.50 %	24

15	782	SEQ ID NO: 8155	1.50 %	24
16	1186	SEQ ID NO: 8156	1.31 %	21
17	910	SEQ ID NO: 8157	1.05 %	16.8
18	128	SEQ ID NO: 8158	0.93 %	15
19	183	SEQ ID NO: 8159	0.93 %	15
20	1069	SEQ ID NO: 8160	0.93 %	15

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	1041	SEQ ID NO: 8161	0.01 %	484.2379773
2	981	SEQ ID NO: 8162	0.00 %	382.536
3	957	SEQ ID NO: 8163	0.00 %	342.4606344
4	896	SEQ ID NO: 8164	0.00 %	232.6931712
5	1173	SEQ ID NO: 8165	0.00 %	201.447432
6	733	SEQ ID NO: 8166	0.00 %	171.86796
7	410	SEQ ID NO: 8167	0.00 %	135.45252
8	786	SEQ ID NO: 8168	0.00 %	119.463012
9	150	SEQ ID NO: 8169	0.00 %	102.17550222
10	1	SEQ ID NO: 8170	0.00 %	94.98737754
11	595	SEQ ID NO: 8171	0.00 %	93.239424
12	1095	SEQ ID NO: 8172	0.00 %	89.41779
13	1166	SEQ ID NO: 8173	0.00 %	87.58584
14	845	SEQ ID NO: 8174	0.00 %	79.642008
15	734	SEQ ID NO: 8175	0.00 %	73.47672
16	802	SEQ ID NO: 8176	0.00 %	71.872056
17	1213	SEQ ID NO: 8177	0.00 %	71.872056
18	105	SEQ ID NO: 8178	0.00 %	50.232
19	939	SEQ ID NO: 8179	0.00 %	49.13352
20	130	SEQ ID NO: 8180	0.00 %	48.732354

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	372	SEQ ID NO: 8181	0.04 %	1896.33528
2	410	SEQ ID NO: 8182	0.02 %	1134.00849744
3	162	SEQ ID NO: 8183	0.01 %	685.3897512
4	1076	SEQ ID NO: 8184	0.01 %	640.90320525
5	1196	SEQ ID NO: 8185	0.01 %	623.742666372

6	353	SEQ ID NO: 8186	0.01 %	446.7384576
7	50	SEQ ID NO: 8187	0.00 %	375.97824
8	733	SEQ ID NO: 8188	0.00 %	271.863864
9	130	SEQ ID NO: 8189	0.00 %	235.6873848
10	415	SEQ ID NO: 8190	0.00 %	185.679
11	297	SEQ ID NO: 8191	0.00 %	177.496704
12	1	SEQ ID NO: 8192	0.00 %	152.42160582
13	56	SEQ ID NO: 8193	0.00 %	110.013876
14	732	SEQ ID NO: 8194	0.00 %	101.0988
15	6	SEQ ID NO: 8195	0.00 %	98.26704
16	261	SEQ ID NO: 8196	0.00 %	91.60164
17	1040	SEQ ID NO: 8197	0.00 %	76.98537
18	928	SEQ ID NO: 8198	0.00 %	71.2908
19	1188	SEQ ID NO: 8199	0.00 %	69.81282
20	1094	SEQ ID NO: 8200	0.00 %	52.5987

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	402	SEQ ID NO: 8201	25 %	9
2	902	SEQ ID NO: 8202	22.5 %	8.1
3	288	SEQ ID NO: 8203	11.11 %	4
4	85	SEQ ID NO: 8204	6.66 %	2.4
5	706	SEQ ID NO: 8205	6.66 %	2.4
6	456	SEQ ID NO: 8206	5.55 %	2
7	920	SEQ ID NO: 8207	5.55 %	2
8	535	SEQ ID NO: 8208	5 %	1.8
9	364	SEQ ID NO: 8209	3.33 %	1.2
10	438	SEQ ID NO: 8210	3.33 %	1.2
11	798	SEQ ID NO: 8211	3.33 %	1.2
12	808	SEQ ID NO: 8212	3.33 %	1.2
13	937	SEQ ID NO: 8213	3.33 %	1.2
14	956	SEQ ID NO: 8214	3.33 %	1.2
15	557	SEQ ID NO: 8215	2.77 %	1
16	1218	SEQ ID NO: 8216	2.77 %	1
17	784	SEQ ID NO: 8217	2.5 %	0.9
18	249	SEQ ID NO: 8218	2.22 %	0.8
19	768	SEQ ID NO: 8219	2.22 %	0.8
20	1178	SEQ ID NO: 8220	2.22 %	0.8

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	38	SEQ ID NO: 8221	13.33 %	4.8
2	807	SEQ ID NO: 8222	12.5 %	4.5
3	100	SEQ ID NO: 8223	11.11 %	4
4	380	SEQ ID NO: 8224	11.11 %	4
5	767	SEQ ID NO: 8225	10 %	3.6
6	533	SEQ ID NO: 8226	8.33 %	3
7	967	SEQ ID NO: 8227	6.66 %	2.4
8	919	SEQ ID NO: 8228	5.55 %	2
9	305	SEQ ID NO: 8229	5 %	1.8
10	211	SEQ ID NO: 8230	3.33 %	1.2
11	511	SEQ ID NO: 8231	3.33 %	1.2
12	1177	SEQ ID NO: 8232	3.33 %	1.2
13	429	SEQ ID NO: 8233	2.77 %	1
14	758	SEQ ID NO: 8234	2.77 %	1
15	797	SEQ ID NO: 8235	2.5 %	0.9
16	255	SEQ ID NO: 8236	2.22 %	0.8
17	986	SEQ ID NO: 8237	2.22 %	0.8
18	1157	SEQ ID NO: 8238	2.22 %	0.8
19	170	SEQ ID NO: 8239	1.66 %	0.6
20	893	SEQ ID NO: 8240	1.66 %	0.6

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	200	SEQ ID NO: 8241	1.48 %	80
2	1243	SEQ ID NO: 8242	1.48 %	80
3	123	SEQ ID NO: 8243	0.74 %	40
4	248	SEQ ID NO: 8244	0.66 %	36
5	1036	SEQ ID NO: 8245	0.66 %	36
6	494	SEQ ID NO: 8246	0.37 %	20
7	495	SEQ ID NO: 8247	0.37 %	20
8	523	SEQ ID NO: 8248	0.37 %	20
9	842	SEQ ID NO: 8249	0.37 %	20
10	932	SEQ ID NO: 8250	0.37 %	20
11	274	SEQ ID NO: 8251	0.33 %	18

12	588	SEQ ID NO: 8252	0.22 %	12
13	656	SEQ ID NO: 8253	0.22 %	12
14	657	SEQ ID NO: 8254	0.22 %	12
15	767	SEQ ID NO: 8255	0.22 %	12
16	911	SEQ ID NO: 8256	0.22 %	12
17	939	SEQ ID NO: 8257	0.22 %	12
18	1007	SEQ ID NO: 8258	0.22 %	12
19	1170	SEQ ID NO: 8259	0.22 %	12
20	1206	SEQ ID NO: 8260	0.22 %	12

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	505	SEQ ID NO: 8261	4.44 %	240
2	312	SEQ ID NO: 8262	3.70 %	200
3	141	SEQ ID NO: 8263	1.11 %	60
4	1006	SEQ ID NO: 8264	0.66 %	36
5	411	SEQ ID NO: 8265	0.44 %	24
6	122	SEQ ID NO: 8266	0.37 %	20
7	134	SEQ ID NO: 8267	0.37 %	20
8	184	SEQ ID NO: 8268	0.37 %	20
9	367	SEQ ID NO: 8269	0.37 %	20
10	402	SEQ ID NO: 8270	0.37 %	20
11	494	SEQ ID NO: 8271	0.37 %	20
12	560	SEQ ID NO: 8272	0.37 %	20
13	626	SEQ ID NO: 8273	0.37 %	20
14	931	SEQ ID NO: 8274	0.37 %	20
15	956	SEQ ID NO: 8275	0.37 %	20
16	1117	SEQ ID NO: 8276	0.37 %	20
17	1169	SEQ ID NO: 8277	0.37 %	20
18	1196	SEQ ID NO: 8278	0.37 %	20
19	247	SEQ ID NO: 8279	0.22 %	12
20	273	SEQ ID NO: 8280	0.22 %	12

Table 17: Epitopes for SEQ ID NO: 6043

HLA A1 - 9 mers	
Maximum possible score using this molecule type	5625

Rank	Start position	Sequence	% of max. score	Score
1	168	SEQ ID NO: 8281	0.2 %	11.25
2	212	SEQ ID NO: 8282	0.08 %	4.5
3	223	SEQ ID NO: 8283	0.08 %	4.5
4	104	SEQ ID NO: 8284	0.04 %	2.5
5	170	SEQ ID NO: 8285	0.04 %	2.5
6	99	SEQ ID NO: 8286	0.04 %	2.25
7	188	SEQ ID NO: 8287	0.02 %	1.35
8	180	SEQ ID NO: 8288	0.02 %	1.25
9	219	SEQ ID NO: 8289	0.02 %	1.25
10	18	SEQ ID NO: 8290	0.01 %	1
11	226	SEQ ID NO: 8291	0.01 %	1
12	98	SEQ ID NO: 8292	0.01 %	0.625
13	151	SEQ ID NO: 8293	0.01 %	0.625
14	10	SEQ ID NO: 8294	0.01 %	0.6
15	13	SEQ ID NO: 8295	0.00 %	0.5
16	32	SEQ ID NO: 8296	0.00 %	0.5
17	70	SEQ ID NO: 8297	0.00 %	0.5
18	78	SEQ ID NO: 8298	0.00 %	0.5
19	82	SEQ ID NO: 8299	0.00 %	0.5
20	145	SEQ ID NO: 8300	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	99	SEQ ID NO: 8301	0.8 %	45
2	223	SEQ ID NO: 8302	0.8 %	45
3	188	SEQ ID NO: 8303	0.48 %	27
4	206	SEQ ID NO: 8304	0.2 %	11.25
5	253	SEQ ID NO: 8305	0.17 %	10
6	174	SEQ ID NO: 8306	0.13 %	7.5
7	97	SEQ ID NO: 8307	0.04 %	2.5
8	257	SEQ ID NO: 8308	0.04 %	2.5
9	179	SEQ ID NO: 8309	0.04 %	2.25
10	162	SEQ ID NO: 8310	0.02 %	1.25
11	196	SEQ ID NO: 8311	0.02 %	1.25
12	219	SEQ ID NO: 8312	0.02 %	1.25
13	18	SEQ ID NO: 8313	0.01 %	1
14	246	SEQ ID NO: 8314	0.01 %	1

15	38	SEQ ID NO: 8315	0.01 %	0.75
16	33	SEQ ID NO: 8316	0.00 %	0.5
17	69	SEQ ID NO: 8317	0.00 %	0.5
18	81	SEQ ID NO: 8318	0.00 %	0.5
19	104	SEQ ID NO: 8319	0.00 %	0.5
20	116	SEQ ID NO: 8320	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	104	SEQ ID NO: 8321	0.98 %	120
2	123	SEQ ID NO: 8322	0.74 %	90
3	82	SEQ ID NO: 8323	0.44 %	54
4	106	SEQ ID NO: 8324	0.11 %	13.5
5	99	SEQ ID NO: 8325	0.08 %	10.8
6	127	SEQ ID NO: 8326	0.08 %	10
7	71	SEQ ID NO: 8327	0.07 %	9
8	1	SEQ ID NO: 8328	0.06 %	8.1
9	113	SEQ ID NO: 8329	0.04 %	6
10	84	SEQ ID NO: 8330	0.03 %	4.5
11	109	SEQ ID NO: 8331	0.03 %	4.05
12	58	SEQ ID NO: 8332	0.02 %	3
13	138	SEQ ID NO: 8333	0.02 %	3
14	44	SEQ ID NO: 8334	0.02 %	2.7
15	81	SEQ ID NO: 8335	0.02 %	2.7
16	226	SEQ ID NO: 8336	0.02 %	2.7
17	184	SEQ ID NO: 8337	0.01 %	1.8
18	102	SEQ ID NO: 8338	0.01 %	1.215
19	39	SEQ ID NO: 8339	0.00 %	1.2
20	234	SEQ ID NO: 8340	0.00 %	0.9

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	99	SEQ ID NO: 8341	1.33 %	162
2	81	SEQ ID NO: 8342	0.44 %	54
3	104	SEQ ID NO: 8343	0.24 %	30
4	51	SEQ ID NO: 8344	0.16 %	20
5	122	SEQ ID NO: 8345	0.11 %	13.5

6	71	SEQ ID NO: 8346	0.07 %	9
7	69	SEQ ID NO: 8347	0.04 %	6
8	223	SEQ ID NO: 8348	0.04 %	5.4
9	84	SEQ ID NO: 8349	0.03 %	4.5
10	63	SEQ ID NO: 8350	0.02 %	3.6
11	138	SEQ ID NO: 8351	0.02 %	3
12	201	SEQ ID NO: 8352	0.01 %	1.8
13	44	SEQ ID NO: 8353	0.01 %	1.35
14	83	SEQ ID NO: 8354	0.01 %	1.35
15	116	SEQ ID NO: 8355	0.00 %	1.2
16	46	SEQ ID NO: 8356	0.00 %	0.9
17	183	SEQ ID NO: 8357	0.00 %	0.81
18	57	SEQ ID NO: 8358	0.00 %	0.6
19	93	SEQ ID NO: 8359	0.00 %	0.6
20	113	SEQ ID NO: 8360	0.00 %	0.6

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	198	SEQ ID NO: 8361	13.15 %	210
2	105	SEQ ID NO: 8362	9.39 %	150
3	210	SEQ ID NO: 8363	4.69 %	75
4	75	SEQ ID NO: 8364	3.15 %	50.4
5	85	SEQ ID NO: 8365	2.63 %	42
6	205	SEQ ID NO: 8366	2.10 %	33.6
7	77	SEQ ID NO: 8367	1.87 %	30
8	158	SEQ ID NO: 8368	0.65 %	10.5
9	103	SEQ ID NO: 8369	0.56 %	9
10	227	SEQ ID NO: 8370	0.55 %	8.8704
11	32	SEQ ID NO: 8371	0.54 %	8.64
12	74	SEQ ID NO: 8372	0.50 %	8
13	131	SEQ ID NO: 8373	0.50 %	8
14	54	SEQ ID NO: 8374	0.46 %	7.5
15	99	SEQ ID NO: 8375	0.45 %	7.2
16	44	SEQ ID NO: 8376	0.37 %	6
17	62	SEQ ID NO: 8377	0.37 %	6
18	87	SEQ ID NO: 8378	0.37 %	6
19	89	SEQ ID NO: 8379	0.37 %	6
20	154	SEQ ID NO: 8380	0.37 %	6

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	Start position	Sequence	% of max. score	Score
1	105	SEQ ID NO: 8381	22.54 %	360
2	204	SEQ ID NO: 8382	17.53 %	280
3	209	SEQ ID NO: 8383	3.13 %	50
4	75	SEQ ID NO: 8384	1.87 %	30
5	85	SEQ ID NO: 8385	1.87 %	30
6	77	SEQ ID NO: 8386	1.12 %	18
7	74	SEQ ID NO: 8387	0.84 %	13.44
8	210	SEQ ID NO: 8388	0.56 %	9
9	226	SEQ ID NO: 8389	0.55 %	8.8704
10	98	SEQ ID NO: 8390	0.54 %	8.64
11	198	SEQ ID NO: 8391	0.46 %	7.5
12	67	SEQ ID NO: 8392	0.45 %	7.2
13	152	SEQ ID NO: 8393	0.43 %	7
14	43	SEQ ID NO: 8394	0.37 %	6
15	63	SEQ ID NO: 8395	0.37 %	6
16	72	SEQ ID NO: 8396	0.37 %	6
17	89	SEQ ID NO: 8397	0.37 %	6
18	101	SEQ ID NO: 8398	0.37 %	6
19	107	SEQ ID NO: 8399	0.37 %	6
20	111	SEQ ID NO: 8400	0.37 %	6

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	Start position	Sequence	% of max. score	Score
1	138	SEQ ID NO: 8401	0.21 %	8532.082944
2	106	SEQ ID NO: 8402	0.10 %	3977.8497792
3	44	SEQ ID NO: 8403	0.03 %	1243.078056
4	71	SEQ ID NO: 8404	0.00 %	348.872832
5	234	SEQ ID NO: 8405	0.00 %	243.432
6	51	SEQ ID NO: 8406	0.00 %	130.26096
7	109	SEQ ID NO: 8407	0.00 %	91.182672
8	81	SEQ ID NO: 8408	0.00 %	73.342584
9	88	SEQ ID NO: 8409	0.00 %	70.386624
10	1	SEQ ID NO: 8410	0.00 %	65.32728732
11	38	SEQ ID NO: 8411	0.00 %	47.876409

12	76	SEQ ID NO: 8412	0.00 %	36.8637882
13	46	SEQ ID NO: 8413	0.00 %	30.889782
14	211	SEQ ID NO: 8414	0.00 %	21.616753941
15	201	SEQ ID NO: 8415	0.00 %	19.657134
16	102	SEQ ID NO: 8416	0.00 %	18.4318941
17	199	SEQ ID NO: 8417	0.00 %	16.496865
18	74	SEQ ID NO: 8418	0.00 %	15.783256167
19	62	SEQ ID NO: 8419	0.00 %	13.9968225
20	99	SEQ ID NO: 8420	0.00 %	10.31851392

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	78	SEQ ID NO: 8421	0.01 %	556.494246
2	138	SEQ ID NO: 8422	0.01 %	395.245972224
3	84	SEQ ID NO: 8423	0.00 %	201.554244
4	71	SEQ ID NO: 8424	0.00 %	143.65707264
5	44	SEQ ID NO: 8425	0.00 %	132.54624
6	76	SEQ ID NO: 8426	0.00 %	84.78671286
7	8	SEQ ID NO: 8427	0.00 %	69.552
8	211	SEQ ID NO: 8428	0.00 %	52.7237901
9	113	SEQ ID NO: 8429	0.00 %	47.99088
10	61	SEQ ID NO: 8430	0.00 %	37.4509575
11	93	SEQ ID NO: 8431	0.00 %	31.24872
12	137	SEQ ID NO: 8432	0.00 %	31.1384304
13	37	SEQ ID NO: 8433	0.00 %	27.531
14	55	SEQ ID NO: 8434	0.00 %	22.9153278
15	98	SEQ ID NO: 8435	0.00 %	22.1063618985
16	108	SEQ ID NO: 8436	0.00 %	21.55457052
17	63	SEQ ID NO: 8437	0.00 %	21.3624
18	45	SEQ ID NO: 8438	0.00 %	19.657134
19	200	SEQ ID NO: 8439	0.00 %	19.657134
20	104	SEQ ID NO: 8440	0.00 %	13.87622016

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	58	SEQ ID NO: 8441	5.55 %	2
2	125	SEQ ID NO: 8442	1.66 %	0.6

3	226	SEQ ID NO: 8443	1.66 %	0.6
4	229	SEQ ID NO: 8444	1.66 %	0.6

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	122	SEQ ID NO: 8445	2.22 %	0.8
2	228	SEQ ID NO: 8446	2.22 %	0.8

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	97	SEQ ID NO: 8447	0.66 %	36
2	86	SEQ ID NO: 8448	0.37 %	20
3	37	SEQ ID NO: 8449	0.33 %	18
4	62	SEQ ID NO: 8450	0.33 %	18
5	32	SEQ ID NO: 8451	0.22 %	12
6	102	SEQ ID NO: 8452	0.22 %	12
7	227	SEQ ID NO: 8453	0.22 %	12
8	53	SEQ ID NO: 8454	0.11 %	6
9	1	SEQ ID NO: 8455	0.07 %	4
10	44	SEQ ID NO: 8456	0.07 %	4
11	56	SEQ ID NO: 8457	0.07 %	4
12	64	SEQ ID NO: 8458	0.07 %	4
13	74	SEQ ID NO: 8459	0.07 %	4
14	76	SEQ ID NO: 8460	0.07 %	4
15	87	SEQ ID NO: 8461	0.07 %	4
16	106	SEQ ID NO: 8462	0.07 %	4
17	131	SEQ ID NO: 8463	0.07 %	4
18	23	SEQ ID NO: 8464	0.03 %	2
19	157	SEQ ID NO: 8465	0.03 %	2
20	166	SEQ ID NO: 8466	0.03 %	2

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	119	SEQ ID NO: 8467	3.33 %	180
2	264	SEQ ID NO: 8468	1.48 %	80
3	98	SEQ ID NO: 8469	0.66 %	36

4	27	SEQ ID NO: 8470	0.37 %	20
5	86	SEQ ID NO: 8471	0.37 %	20
6	31	SEQ ID NO: 8472	0.22 %	12
7	63	SEQ ID NO: 8473	0.22 %	12
8	96	SEQ ID NO: 8474	0.22 %	12
9	101	SEQ ID NO: 8475	0.22 %	12
10	226	SEQ ID NO: 8476	0.22 %	12
11	157	SEQ ID NO: 8477	0.14 %	8
12	176	SEQ ID NO: 8478	0.14 %	8
13	238	SEQ ID NO: 8479	0.14 %	8
14	36	SEQ ID NO: 8480	0.11 %	6
15	53	SEQ ID NO: 8481	0.11 %	6
16	61	SEQ ID NO: 8482	0.11 %	6
17	3	SEQ ID NO: 8483	0.07 %	4
18	40	SEQ ID NO: 8484	0.07 %	4
19	55	SEQ ID NO: 8485	0.07 %	4
20	74	SEQ ID NO: 8486	0.07 %	4

Table 18: Epitopes for SEQ ID NO: 6044

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	69	SEQ ID NO: 8487	0.04 %	2.5
2	89	SEQ ID NO: 8488	0.02 %	1.5
3	141	SEQ ID NO: 8489	0.01 %	1
4	113	SEQ ID NO: 8490	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	21	SEQ ID NO: 8491	0.02 %	1.5
2	88	SEQ ID NO: 8492	0.02 %	1.5
3	8	SEQ ID NO: 8493	0.02 %	1.25
4	31	SEQ ID NO: 8494	0.00 %	0.5
5	112	SEQ ID NO: 8495	0.00 %	0.5

HLA A3 - 9 mers

Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	60	SEQ ID NO: 8496	1.23 %	150
2	77	SEQ ID NO: 8497	1.11 %	135
3	141	SEQ ID NO: 8498	0.49 %	60
4	95	SEQ ID NO: 8499	0.32 %	40
5	128	SEQ ID NO: 8500	0.08 %	10
6	113	SEQ ID NO: 8501	0.04 %	6
7	69	SEQ ID NO: 8502	0.01 %	2
8	22	SEQ ID NO: 8503	0.01 %	1.8
9	42	SEQ ID NO: 8504	0.01 %	1.8
10	78	SEQ ID NO: 8505	0.00 %	1.2
11	32	SEQ ID NO: 8506	0.00 %	1
12	54	SEQ ID NO: 8507	0.00 %	0.9
13	74	SEQ ID NO: 8508	0.00 %	0.9
14	28	SEQ ID NO: 8509	0.00 %	0.6
15	36	SEQ ID NO: 8510	0.00 %	0.6
16	48	SEQ ID NO: 8511	0.00 %	0.6
17	118	SEQ ID NO: 8512	0.00 %	0.6
18	4	SEQ ID NO: 8513	0.00 %	0.5

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	94	SEQ ID NO: 8514	0.49 %	60
2	48	SEQ ID NO: 8515	0.16 %	20
3	128	SEQ ID NO: 8516	0.16 %	20
4	60	SEQ ID NO: 8517	0.12 %	15
5	127	SEQ ID NO: 8518	0.12 %	15
6	25	SEQ ID NO: 8519	0.04 %	6
7	95	SEQ ID NO: 8520	0.04 %	6
8	141	SEQ ID NO: 8521	0.04 %	6
9	41	SEQ ID NO: 8522	0.04 %	5.4
10	77	SEQ ID NO: 8523	0.04 %	5.4
11	116	SEQ ID NO: 8524	0.04 %	5.4
12	91	SEQ ID NO: 8525	0.03 %	4
13	4	SEQ ID NO: 8526	0.01 %	2
14	112	SEQ ID NO: 8527	0.01 %	1.8
15	113	SEQ ID NO: 8528	0.01 %	1.35

16	12	SEQ ID NO: 8529	0.00 %	1.2
17	31	SEQ ID NO: 8530	0.00 %	1
18	32	SEQ ID NO: 8531	0.00 %	1
19	15	SEQ ID NO: 8532	0.00 %	0.9
20	27	SEQ ID NO: 8533	0.00 %	0.9

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	61	SEQ ID NO: 8534	14.46 %	231
2	16	SEQ ID NO: 8535	3.13 %	50
3	120	SEQ ID NO: 8536	1.87 %	30
4	41	SEQ ID NO: 8537	0.60 %	9.6
5	71	SEQ ID NO: 8538	0.45 %	7.2
6	21	SEQ ID NO: 8539	0.37 %	6
7	53	SEQ ID NO: 8540	0.37 %	6
8	65	SEQ ID NO: 8541	0.37 %	6
9	121	SEQ ID NO: 8542	0.37 %	6
10	74	SEQ ID NO: 8543	0.36 %	5.76
11	20	SEQ ID NO: 8544	0.35 %	5.6
12	79	SEQ ID NO: 8545	0.35 %	5.6
13	105	SEQ ID NO: 8546	0.33 %	5.28
14	48	SEQ ID NO: 8547	0.30 %	4.8
15	88	SEQ ID NO: 8548	0.30 %	4.8
16	106	SEQ ID NO: 8549	0.30 %	4.8
17	37	SEQ ID NO: 8550	0.27 %	4.4
18	70	SEQ ID NO: 8551	0.27 %	4.4
19	18	SEQ ID NO: 8552	0.25 %	4
20	57	SEQ ID NO: 8553	0.22 %	3.6

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	120	SEQ ID NO: 8554	1.87 %	30
2	73	SEQ ID NO: 8555	0.54 %	8.64
3	19	SEQ ID NO: 8556	0.52 %	8.4
4	78	SEQ ID NO: 8557	0.52 %	8.4
5	104	SEQ ID NO: 8558	0.49 %	7.92
6	61	SEQ ID NO: 8559	0.46 %	7.5

7	47	SEQ ID NO: 8560	0.45 %	7.2
8	36	SEQ ID NO: 8561	0.41 %	6.6
9	52	SEQ ID NO: 8562	0.37 %	6
10	64	SEQ ID NO: 8563	0.30 %	4.8
11	70	SEQ ID NO: 8564	0.30 %	4.8
12	105	SEQ ID NO: 8565	0.30 %	4.8
13	123	SEQ ID NO: 8566	0.30 %	4.8
14	69	SEQ ID NO: 8567	0.27 %	4.4
15	20	SEQ ID NO: 8568	0.25 %	4
16	66	SEQ ID NO: 8569	0.25 %	4
17	83	SEQ ID NO: 8570	0.25 %	4
18	86	SEQ ID NO: 8571	0.25 %	4
19	101	SEQ ID NO: 8572	0.25 %	4
20	119	SEQ ID NO: 8573	0.25 %	4

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	62	SEQ ID NO: 8574	0.00 %	136.1646
2	85	SEQ ID NO: 8575	0.00 %	69.6969
3	47	SEQ ID NO: 8576	0.00 %	60.153786
4	121	SEQ ID NO: 8577	0.00 %	52.5182736
5	74	SEQ ID NO: 8578	0.00 %	49.13352
6	23	SEQ ID NO: 8579	0.00 %	21.99582
7	78	SEQ ID NO: 8580	0.00 %	19.42488
8	114	SEQ ID NO: 8581	0.00 %	14.6900655
9	4	SEQ ID NO: 8582	0.00 %	11.304684
10	79	SEQ ID NO: 8583	0.00 %	8.4687081
11	122	SEQ ID NO: 8584	0.00 %	6.0996
12	100	SEQ ID NO: 8585	0.00 %	5.382
13	105	SEQ ID NO: 8586	0.00 %	4.981593
14	25	SEQ ID NO: 8587	0.00 %	4.968
15	115	SEQ ID NO: 8588	0.00 %	4.966482
16	24	SEQ ID NO: 8589	0.00 %	4.4815221585
17	111	SEQ ID NO: 8590	0.00 %	4.128201
18	94	SEQ ID NO: 8591	0.00 %	3.67632
19	34	SEQ ID NO: 8592	0.00 %	3.47553
20	12	SEQ ID NO: 8593	0.00 %	3.30993

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	77	SEQ ID NO: 8594	0.00 %	147.97188
2	62	SEQ ID NO: 8595	0.00 %	143.59176
3	113	SEQ ID NO: 8596	0.00 %	106.83684
4	78	SEQ ID NO: 8597	0.00 %	83.526984
5	86	SEQ ID NO: 8598	0.00 %	83.526984
6	74	SEQ ID NO: 8599	0.00 %	69.552
7	121	SEQ ID NO: 8600	0.00 %	61.06776
8	12	SEQ ID NO: 8601	0.00 %	50.232
9	44	SEQ ID NO: 8602	0.00 %	26.082
10	4	SEQ ID NO: 8603	0.00 %	18.3816
11	0	SEQ ID NO: 8604	0.00 %	17.38386
12	72	SEQ ID NO: 8605	0.00 %	17.1396
13	22	SEQ ID NO: 8606	0.00 %	16.21914
14	122	SEQ ID NO: 8607	0.00 %	14.02908
15	64	SEQ ID NO: 8608	0.00 %	11.161854
16	46	SEQ ID NO: 8609	0.00 %	10.34586
17	54	SEQ ID NO: 8610	0.00 %	8.846145
18	47	SEQ ID NO: 8611	0.00 %	7.575080337
19	131	SEQ ID NO: 8612	0.00 %	7.452
20	114	SEQ ID NO: 8613	0.00 %	6.735366

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	69	SEQ ID NO: 8614	5.55 %	2
2	22	SEQ ID NO: 8615	5 %	1.8
3	77	SEQ ID NO: 8616	5 %	1.8
4	141	SEQ ID NO: 8617	3.33 %	1.2
5	60	SEQ ID NO: 8618	2.22 %	0.8
6	95	SEQ ID NO: 8619	2.22 %	0.8
7	36	SEQ ID NO: 8620	1.66 %	0.6

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	41	SEQ ID NO: 8621	3.33 %	1.2

2	68	SEQ ID NO: 8622	3.33 %	1.2
3	94	SEQ ID NO: 8623	3.33 %	1.2
4	31	SEQ ID NO: 8624	2.77 %	1
5	127	SEQ ID NO: 8625	2.5 %	0.9

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	48	SEQ ID NO: 8626	0.74 %	40
2	20	SEQ ID NO: 8627	0.37 %	20
3	121	SEQ ID NO: 8628	0.33 %	18
4	18	SEQ ID NO: 8629	0.07 %	4
5	21	SEQ ID NO: 8630	0.07 %	4
6	37	SEQ ID NO: 8631	0.07 %	4
7	41	SEQ ID NO: 8632	0.07 %	4
8	53	SEQ ID NO: 8633	0.07 %	4
9	65	SEQ ID NO: 8634	0.07 %	4
10	70	SEQ ID NO: 8635	0.07 %	4
11	71	SEQ ID NO: 8636	0.07 %	4
12	74	SEQ ID NO: 8637	0.07 %	4
13	79	SEQ ID NO: 8638	0.07 %	4
14	88	SEQ ID NO: 8639	0.07 %	4
15	105	SEQ ID NO: 8640	0.07 %	4
16	106	SEQ ID NO: 8641	0.07 %	4
17	124	SEQ ID NO: 8642	0.07 %	4
18	1	SEQ ID NO: 8643	0.03 %	2
19	120	SEQ ID NO: 8644	0.03 %	1.8
20	11	SEQ ID NO: 8645	0.02 %	1.2

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	66	SEQ ID NO: 8646	1.48 %	80
2	123	SEQ ID NO: 8647	0.74 %	40
3	20	SEQ ID NO: 8648	0.37 %	20
4	64	SEQ ID NO: 8649	0.22 %	12
5	119	SEQ ID NO: 8650	0.11 %	6
6	54	SEQ ID NO: 8651	0.09 %	5
7	19	SEQ ID NO: 8652	0.07 %	4

8	36	SEQ ID NO: 8653	0.07 %	4
9	47	SEQ ID NO: 8654	0.07 %	4
10	52	SEQ ID NO: 8655	0.07 %	4
11	69	SEQ ID NO: 8656	0.07 %	4
12	70	SEQ ID NO: 8657	0.07 %	4
13	73	SEQ ID NO: 8658	0.07 %	4
14	78	SEQ ID NO: 8659	0.07 %	4
15	83	SEQ ID NO: 8660	0.07 %	4
16	86	SEQ ID NO: 8661	0.07 %	4
17	101	SEQ ID NO: 8662	0.07 %	4
18	104	SEQ ID NO: 8663	0.07 %	4
19	105	SEQ ID NO: 8664	0.07 %	4
20	15	SEQ ID NO: 8665	0.03 %	2

Table 19: Epitopes for SEQ ID NO: 6045

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	4	SEQ ID NO: 8666	0.02 %	1.35
2	66	SEQ ID NO: 8667	0.02 %	1.35
3	33	SEQ ID NO: 8668	0.02 %	1.25
4	44	SEQ ID NO: 8669	0.01 %	1
5	50	SEQ ID NO: 8670	0.01 %	1
6	14	SEQ ID NO: 8671	0.01 %	0.75
7	48	SEQ ID NO: 8672	0.01 %	0.75
8	11	SEQ ID NO: 8673	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	4	SEQ ID NO: 8674	0.12 %	6.75
2	66	SEQ ID NO: 8675	0.12 %	6.75
3	10	SEQ ID NO: 8676	0.00 %	0.5
4	28	SEQ ID NO: 8677	0.00 %	0.5
5	32	SEQ ID NO: 8678	0.00 %	0.5
6	47	SEQ ID NO: 8679	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	Start position	Sequence	% of max. score	Score
1	17	SEQ ID NO: 8680	0.24 %	30
2	44	SEQ ID NO: 8681	0.07 %	9
3	19	SEQ ID NO: 8682	0.06 %	8.1
4	50	SEQ ID NO: 8683	0.04 %	5.4
5	29	SEQ ID NO: 8684	0.03 %	4
6	52	SEQ ID NO: 8685	0.02 %	3.24
7	54	SEQ ID NO: 8686	0.02 %	3
8	11	SEQ ID NO: 8687	0.01 %	1.8
9	37	SEQ ID NO: 8688	0.01 %	1.8
10	25	SEQ ID NO: 8689	0.01 %	1.35
11	10	SEQ ID NO: 8690	0.00 %	0.9
12	16	SEQ ID NO: 8691	0.00 %	0.9
13	35	SEQ ID NO: 8692	0.00 %	0.6

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	Start position	Sequence	% of max. score	Score
1	49	SEQ ID NO: 8693	0.44 %	54
2	17	SEQ ID NO: 8694	0.22 %	27
3	10	SEQ ID NO: 8695	0.14 %	18
4	16	SEQ ID NO: 8696	0.07 %	9
5	32	SEQ ID NO: 8697	0.04 %	6
6	19	SEQ ID NO: 8698	0.01 %	1.8
7	29	SEQ ID NO: 8699	0.00 %	1.2
8	23	SEQ ID NO: 8700	0.00 %	0.9
9	26	SEQ ID NO: 8701	0.00 %	0.9

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	Start position	Sequence	% of max. score	Score
1	18	SEQ ID NO: 8702	1.87 %	30
2	24	SEQ ID NO: 8703	0.65 %	10.5
3	9	SEQ ID NO: 8704	0.52 %	8.4
4	12	SEQ ID NO: 8705	0.52 %	8.4
5	28	SEQ ID NO: 8706	0.52 %	8.4
6	42	SEQ ID NO: 8707	0.52 %	8.4

7	57	SEQ ID NO: 8708	0.52 %	8.4
8	66	SEQ ID NO: 8709	0.52 %	8.4
9	55	SEQ ID NO: 8710	0.51 %	8.25
10	0	SEQ ID NO: 8711	0.48 %	7.7
11	22	SEQ ID NO: 8712	0.45 %	7.2
12	10	SEQ ID NO: 8713	0.37 %	6
13	25	SEQ ID NO: 8714	0.37 %	6
14	30	SEQ ID NO: 8715	0.37 %	6
15	19	SEQ ID NO: 8716	0.35 %	5.6
16	40	SEQ ID NO: 8717	0.31 %	5
17	3	SEQ ID NO: 8718	0.30 %	4.8
18	65	SEQ ID NO: 8719	0.30 %	4.8
19	14	SEQ ID NO: 8720	0.27 %	4.32
20	56	SEQ ID NO: 8721	0.25 %	4

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	55	SEQ ID NO: 8722	18.78 %	300
2	18	SEQ ID NO: 8723	2.63 %	42
3	21	SEQ ID NO: 8724	2.25 %	36
4	2	SEQ ID NO: 8725	1.87 %	30
5	24	SEQ ID NO: 8726	1.87 %	30
6	11	SEQ ID NO: 8727	0.52 %	8.4
7	40	SEQ ID NO: 8728	0.52 %	8.4
8	65	SEQ ID NO: 8729	0.42 %	6.72
9	9	SEQ ID NO: 8730	0.37 %	6
10	8	SEQ ID NO: 8731	0.35 %	5.6
11	27	SEQ ID NO: 8732	0.35 %	5.6
12	41	SEQ ID NO: 8733	0.35 %	5.6
13	57	SEQ ID NO: 8734	0.31 %	5
14	17	SEQ ID NO: 8735	0.25 %	4
15	29	SEQ ID NO: 8736	0.25 %	4
16	64	SEQ ID NO: 8737	0.25 %	4
17	16	SEQ ID NO: 8738	0.22 %	3.6
18	10	SEQ ID NO: 8739	0.18 %	3
19	13	SEQ ID NO: 8740	0.18 %	2.88
20	23	SEQ ID NO: 8741	0.08 %	1.4

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	Start position	Sequence	% of max. score	Score
1	19	SEQ ID NO: 8742	0.03 %	1310.8823136
2	15	SEQ ID NO: 8743	0.02 %	1082.4143022
3	16	SEQ ID NO: 8744	0.02 %	1040.33238624
4	49	SEQ ID NO: 8745	0.00 %	382.536
5	25	SEQ ID NO: 8746	0.00 %	342.863529264
6	56	SEQ ID NO: 8747	0.00 %	63.28397376
7	12	SEQ ID NO: 8748	0.00 %	40.19736105
8	10	SEQ ID NO: 8749	0.00 %	21.3624
9	22	SEQ ID NO: 8750	0.00 %	19.7762418
10	26	SEQ ID NO: 8751	0.00 %	12.6684
11	20	SEQ ID NO: 8752	0.00 %	11.544666
12	37	SEQ ID NO: 8753	0.00 %	10.4328
13	32	SEQ ID NO: 8754	0.00 %	8.4456
14	23	SEQ ID NO: 8755	0.00 %	6.2888049
15	47	SEQ ID NO: 8756	0.00 %	6.0858
16	3	SEQ ID NO: 8757	0.00 %	4.582929078
17	18	SEQ ID NO: 8758	0.00 %	4.4855150505
18	28	SEQ ID NO: 8759	0.00 %	4.2923589
19	62	SEQ ID NO: 8760	0.00 %	2.88098391
20	27	SEQ ID NO: 8761	0.00 %	1.699677

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	Start position	Sequence	% of max. score	Score
1	17	SEQ ID NO: 8762	0.16 %	6459.14167272
2	19	SEQ ID NO: 8763	0.01 %	607.88448
3	25	SEQ ID NO: 8764	0.00 %	126.83304
4	11	SEQ ID NO: 8765	0.00 %	63.16728165
5	15	SEQ ID NO: 8766	0.00 %	53.54651988
6	37	SEQ ID NO: 8767	0.00 %	28.51632
7	14	SEQ ID NO: 8768	0.00 %	21.8247414
8	29	SEQ ID NO: 8769	0.00 %	21.3624
9	26	SEQ ID NO: 8770	0.00 %	19.42488
10	3	SEQ ID NO: 8771	0.00 %	17.2167282
11	48	SEQ ID NO: 8772	0.00 %	15.7068219
12	12	SEQ ID NO: 8773	0.00 %	9.8581266

13	27	SEQ ID NO: 8774	0.00 %	7.3086111
14	39	SEQ ID NO: 8775	0.00 %	7.10976
15	23	SEQ ID NO: 8776	0.00 %	5.7419523
16	22	SEQ ID NO: 8777	0.00 %	4.599126
17	45	SEQ ID NO: 8778	0.00 %	2.5495155
18	31	SEQ ID NO: 8779	0.00 %	2.52747
19	52	SEQ ID NO: 8780	0.00 %	2.383605
20	20	SEQ ID NO: 8781	0.00 %	2.332847151

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	44	SEQ ID NO: 8782	3.33 %	1.2

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	3	SEQ ID NO: 8783	0.37 %	20
2	12	SEQ ID NO: 8784	0.37 %	20
3	22	SEQ ID NO: 8785	0.37 %	20
4	56	SEQ ID NO: 8786	0.37 %	20
5	30	SEQ ID NO: 8787	0.22 %	12
6	9	SEQ ID NO: 8788	0.07 %	4
7	10	SEQ ID NO: 8789	0.07 %	4
8	19	SEQ ID NO: 8790	0.07 %	4
9	25	SEQ ID NO: 8791	0.07 %	4
10	28	SEQ ID NO: 8792	0.07 %	4
11	42	SEQ ID NO: 8793	0.07 %	4
12	65	SEQ ID NO: 8794	0.07 %	4
13	35	SEQ ID NO: 8795	0.05 %	3
14	66	SEQ ID NO: 8796	0.02 %	1.2
15	15	SEQ ID NO: 8797	0.01 %	1
16	47	SEQ ID NO: 8798	0.01 %	1
17	20	SEQ ID NO: 8799	0.01 %	0.6
18	23	SEQ ID NO: 8800	0.00 %	0.5

19	27	SEQ ID NO: 8801	0.00 %	0.5

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	Start position	Sequence	% of max. score	Score
1	27	SEQ ID NO: 8802	0.37 %	20
2	8	SEQ ID NO: 8803	0.07 %	4
3	9	SEQ ID NO: 8804	0.07 %	4
4	11	SEQ ID NO: 8805	0.07 %	4
5	17	SEQ ID NO: 8806	0.07 %	4
6	29	SEQ ID NO: 8807	0.07 %	4
7	41	SEQ ID NO: 8808	0.07 %	4
8	52	SEQ ID NO: 8809	0.07 %	4
9	64	SEQ ID NO: 8810	0.07 %	4
10	65	SEQ ID NO: 8811	0.07 %	4
11	3	SEQ ID NO: 8812	0.03 %	2
12	23	SEQ ID NO: 8813	0.03 %	2
13	21	SEQ ID NO: 8814	0.02 %	1.2
14	15	SEQ ID NO: 8815	0.01 %	1
15	35	SEQ ID NO: 8816	0.01 %	0.6
16	39	SEQ ID NO: 8817	0.01 %	0.6
17	12	SEQ ID NO: 8818	0.00 %	0.5
18	22	SEQ ID NO: 8819	0.00 %	0.5
19	45	SEQ ID NO: 8820	0.00 %	0.5

Table 20: Epitopes for SEQ ID NO: 6046

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	Start position	Sequence	% of max. score	Score
1	186	SEQ ID NO: 8821	2.22 %	125
2	156	SEQ ID NO: 8822	0.88 %	50
3	14	SEQ ID NO: 8823	0.08 %	4.5
4	0	SEQ ID NO: 8824	0.04 %	2.5
5	29	SEQ ID NO: 8825	0.04 %	2.5
6	85	SEQ ID NO: 8826	0.04 %	2.5
7	168	SEQ ID NO: 8827	0.04 %	2.5
8	133	SEQ ID NO: 8828	0.02 %	1.35

9	111	SEQ ID NO: 8829	0.02 %	1.125
10	61	SEQ ID NO: 8830	0.01 %	1
11	7	SEQ ID NO: 8831	0.01 %	0.9
12	131	SEQ ID NO: 8832	0.01 %	0.9
13	211	SEQ ID NO: 8833	0.01 %	0.625
14	4	SEQ ID NO: 8834	0.00 %	0.5
15	43	SEQ ID NO: 8835	0.00 %	0.5
16	95	SEQ ID NO: 8836	0.00 %	0.5
17	136	SEQ ID NO: 8837	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	133	SEQ ID NO: 8838	0.04 %	2.7
2	84	SEQ ID NO: 8839	0.04 %	2.5
3	167	SEQ ID NO: 8840	0.04 %	2.5
4	186	SEQ ID NO: 8841	0.04 %	2.5
5	131	SEQ ID NO: 8842	0.04 %	2.25
6	14	SEQ ID NO: 8843	0.03 %	1.8
7	205	SEQ ID NO: 8844	0.02 %	1.25
8	111	SEQ ID NO: 8845	0.02 %	1.125
9	60	SEQ ID NO: 8846	0.01 %	1
10	188	SEQ ID NO: 8847	0.01 %	0.75
11	211	SEQ ID NO: 8848	0.01 %	0.625
12	26	SEQ ID NO: 8849	0.00 %	0.5
13	94	SEQ ID NO: 8850	0.00 %	0.5
14	135	SEQ ID NO: 8851	0.00 %	0.5
15	168	SEQ ID NO: 8852	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	43	SEQ ID NO: 8853	0.24 %	30
2	90	SEQ ID NO: 8854	0.14 %	18
3	148	SEQ ID NO: 8855	0.09 %	12
4	4	SEQ ID NO: 8856	0.05 %	6.75
5	24	SEQ ID NO: 8857	0.04 %	6
6	19	SEQ ID NO: 8858	0.04 %	5.4
7	136	SEQ ID NO: 8859	0.04 %	5.4

8	54	SEQ ID NO: 8860	0.03 %	4.5
9	32	SEQ ID NO: 8861	0.03 %	4
10	14	SEQ ID NO: 8862	0.02 %	3.6
11	59	SEQ ID NO: 8863	0.02 %	3.6
12	88	SEQ ID NO: 8864	0.02 %	3
13	87	SEQ ID NO: 8865	0.02 %	2.7
14	29	SEQ ID NO: 8866	0.01 %	1.8
15	48	SEQ ID NO: 8867	0.01 %	1.8
16	115	SEQ ID NO: 8868	0.01 %	1.8
17	186	SEQ ID NO: 8869	0.01 %	1.8
18	106	SEQ ID NO: 8870	0.01 %	1.5
19	53	SEQ ID NO: 8871	0.01 %	1.35
20	173	SEQ ID NO: 8872	0.00 %	1.2

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	24	SEQ ID NO: 8873	0.22 %	27
2	54	SEQ ID NO: 8874	0.18 %	22.5
3	135	SEQ ID NO: 8875	0.08 %	10.8
4	51	SEQ ID NO: 8876	0.07 %	9
5	13	SEQ ID NO: 8877	0.06 %	8.1
6	26	SEQ ID NO: 8878	0.04 %	6
7	31	SEQ ID NO: 8879	0.04 %	6
8	90	SEQ ID NO: 8880	0.04 %	6
9	43	SEQ ID NO: 8881	0.03 %	4.5
10	19	SEQ ID NO: 8882	0.03 %	4.05
11	169	SEQ ID NO: 8883	0.02 %	3
12	87	SEQ ID NO: 8884	0.02 %	2.7
13	84	SEQ ID NO: 8885	0.01 %	1.8
14	88	SEQ ID NO: 8886	0.01 %	1.8
15	94	SEQ ID NO: 8887	0.01 %	1.8
16	64	SEQ ID NO: 8888	0.00 %	1.2
17	131	SEQ ID NO: 8889	0.00 %	1.2
18	99	SEQ ID NO: 8890	0.00 %	1
19	53	SEQ ID NO: 8891	0.00 %	0.9
20	85	SEQ ID NO: 8892	0.00 %	0.9

HLA A24 - 9 mers

Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	196	SEQ ID NO: 8893	27.55 %	440
2	44	SEQ ID NO: 8894	18.78 %	300
3	36	SEQ ID NO: 8895	12.52 %	200
4	92	SEQ ID NO: 8896	12.52 %	200
5	109	SEQ ID NO: 8897	2.70 %	43.2
6	25	SEQ ID NO: 8898	1.87 %	30
7	93	SEQ ID NO: 8899	1.12 %	18
8	12	SEQ ID NO: 8900	0.75 %	12
9	123	SEQ ID NO: 8901	0.70 %	11.2
10	7	SEQ ID NO: 8902	0.64 %	10.368
11	17	SEQ ID NO: 8903	0.52 %	8.4
12	139	SEQ ID NO: 8904	0.52 %	8.4
13	193	SEQ ID NO: 8905	0.46 %	7.5
14	6	SEQ ID NO: 8906	0.45 %	7.2
15	19	SEQ ID NO: 8907	0.45 %	7.2
16	110	SEQ ID NO: 8908	0.45 %	7.2
17	114	SEQ ID NO: 8909	0.45 %	7.2
18	210	SEQ ID NO: 8910	0.45 %	7.2
19	46	SEQ ID NO: 8911	0.42 %	6.72
20	52	SEQ ID NO: 8912	0.37 %	6

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	92	SEQ ID NO: 8913	7.51 %	120
2	42	SEQ ID NO: 8914	2.63 %	42
3	109	SEQ ID NO: 8915	2.25 %	36
4	23	SEQ ID NO: 8916	1.87 %	30
5	34	SEQ ID NO: 8917	0.75 %	12
6	6	SEQ ID NO: 8918	0.64 %	10.368
7	45	SEQ ID NO: 8919	0.63 %	10.08
8	196	SEQ ID NO: 8920	0.62 %	10
9	44	SEQ ID NO: 8921	0.56 %	9
10	40	SEQ ID NO: 8922	0.55 %	8.8
11	62	SEQ ID NO: 8923	0.46 %	7.5
12	193	SEQ ID NO: 8924	0.46 %	7.5
13	18	SEQ ID NO: 8925	0.45 %	7.2

14	113	SEQ ID NO: 8926	0.45 %	7.2
15	56	SEQ ID NO: 8927	0.37 %	6
16	176	SEQ ID NO: 8928	0.37 %	6
17	16	SEQ ID NO: 8929	0.35 %	5.6
18	138	SEQ ID NO: 8930	0.35 %	5.6
19	127	SEQ ID NO: 8931	0.33 %	5.28
20	36	SEQ ID NO: 8932	0.31 %	5

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	13	SEQ ID NO: 8933	0.04 %	1793.676528
2	87	SEQ ID NO: 8934	0.03 %	1415.3832
3	24	SEQ ID NO: 8935	0.01 %	618.0996816
4	19	SEQ ID NO: 8936	0.00 %	223.23708
5	12	SEQ ID NO: 8937	0.00 %	210.36400875
6	51	SEQ ID NO: 8938	0.00 %	198.30859992
7	53	SEQ ID NO: 8939	0.00 %	194.477328
8	88	SEQ ID NO: 8940	0.00 %	180.58536756
9	106	SEQ ID NO: 8941	0.00 %	169.74828
10	54	SEQ ID NO: 8942	0.00 %	70.09848
11	59	SEQ ID NO: 8943	0.00 %	43.42032
12	94	SEQ ID NO: 8944	0.00 %	41.792058
13	20	SEQ ID NO: 8945	0.00 %	37.46088108
14	63	SEQ ID NO: 8946	0.00 %	35.73520902
15	22	SEQ ID NO: 8947	0.00 %	20.5916435109
16	47	SEQ ID NO: 8948	0.00 %	12.233222865
17	66	SEQ ID NO: 8949	0.00 %	12.2199
18	56	SEQ ID NO: 8950	0.00 %	11.486706
19	67	SEQ ID NO: 8951	0.00 %	6.416172
20	117	SEQ ID NO: 8952	0.00 %	5.827464

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	43	SEQ ID NO: 8953	0.10 %	3977.8497792
2	24	SEQ ID NO: 8954	0.02 %	836.2525104
3	51	SEQ ID NO: 8955	0.02 %	815.616432
4	49	SEQ ID NO: 8956	0.01 %	660.3245145

5	19	SEQ ID NO: 8957	0.00 %	251.837856
6	59	SEQ ID NO: 8958	0.00 %	159.9696
7	12	SEQ ID NO: 8959	0.00 %	155.245377
8	45	SEQ ID NO: 8960	0.00 %	141.1974531
9	21	SEQ ID NO: 8961	0.00 %	117.22672269
10	53	SEQ ID NO: 8962	0.00 %	84.55536
11	87	SEQ ID NO: 8963	0.00 %	65.5671672
12	13	SEQ ID NO: 8964	0.00 %	64.88888616
13	153	SEQ ID NO: 8965	0.00 %	49.13352
14	178	SEQ ID NO: 8966	0.00 %	26.082
15	18	SEQ ID NO: 8967	0.00 %	24.802259691
16	116	SEQ ID NO: 8968	0.00 %	21.5616168
17	65	SEQ ID NO: 8969	0.00 %	20.77383
18	86	SEQ ID NO: 8970	0.00 %	15.7068219
19	27	SEQ ID NO: 8971	0.00 %	12.3159135
20	46	SEQ ID NO: 8972	0.00 %	11.45624789925

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	4	SEQ ID NO: 8973	12.5 %	4.5
2	136	SEQ ID NO: 8974	3.33 %	1.2
3	156	SEQ ID NO: 8975	3.33 %	1.2
4	140	SEQ ID NO: 8976	1.66 %	0.6

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	169	SEQ ID NO: 8977	5.55 %	2
2	94	SEQ ID NO: 8978	3.33 %	1.2

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	146	SEQ ID NO: 8979	0.74 %	40
2	154	SEQ ID NO: 8980	0.74 %	40
3	80	SEQ ID NO: 8981	0.66 %	36
4	139	SEQ ID NO: 8982	0.33 %	18
5	83	SEQ ID NO: 8983	0.22 %	12

6	209	SEQ ID NO: 8984	0.22 %	12
7	7	SEQ ID NO: 8985	0.11 %	6
8	3	SEQ ID NO: 8986	0.07 %	4
9	6	SEQ ID NO: 8987	0.07 %	4
10	12	SEQ ID NO: 8988	0.07 %	4
11	19	SEQ ID NO: 8989	0.07 %	4
12	24	SEQ ID NO: 8990	0.07 %	4
13	38	SEQ ID NO: 8991	0.07 %	4
14	46	SEQ ID NO: 8992	0.07 %	4
15	56	SEQ ID NO: 8993	0.07 %	4
16	110	SEQ ID NO: 8994	0.07 %	4
17	114	SEQ ID NO: 8995	0.07 %	4
18	123	SEQ ID NO: 8996	0.07 %	4
19	129	SEQ ID NO: 8997	0.07 %	4
20	166	SEQ ID NO: 8998	0.07 %	4

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	56	SEQ ID NO: 8999	1.48 %	80
2	40	SEQ ID NO: 9000	0.74 %	40
3	127	SEQ ID NO: 9001	0.74 %	40
4	170	SEQ ID NO: 9002	0.74 %	40
5	140	SEQ ID NO: 9003	0.27 %	15
6	35	SEQ ID NO: 9004	0.22 %	12
7	79	SEQ ID NO: 9005	0.22 %	12
8	82	SEQ ID NO: 9006	0.22 %	12
9	208	SEQ ID NO: 9007	0.22 %	12
10	209	SEQ ID NO: 9008	0.22 %	12
11	80	SEQ ID NO: 9009	0.16 %	9
12	129	SEQ ID NO: 9010	0.14 %	8
13	138	SEQ ID NO: 9011	0.11 %	6
14	73	SEQ ID NO: 9012	0.09 %	5
15	2	SEQ ID NO: 9013	0.07 %	4
16	5	SEQ ID NO: 9014	0.07 %	4
17	6	SEQ ID NO: 9015	0.07 %	4
18	16	SEQ ID NO: 9016	0.07 %	4
19	18	SEQ ID NO: 9017	0.07 %	4
20	24	SEQ ID NO: 9018	0.07 %	4

Table 21: Epitopes for SEQ ID NO: 6047

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	53	SEQ ID NO: 9019	2 %	112.5
2	10	SEQ ID NO: 9020	0.08 %	4.5
3	33	SEQ ID NO: 9021	0.02 %	1.5
4	3	SEQ ID NO: 9022	0.00 %	0.5
5	27	SEQ ID NO: 9023	0.00 %	0.5
6	29	SEQ ID NO: 9024	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	10	SEQ ID NO: 9025	0.8 %	45
2	52	SEQ ID NO: 9026	0.2 %	11.25
3	50	SEQ ID NO: 9027	0.04 %	2.5
4	32	SEQ ID NO: 9028	0.02 %	1.5
5	48	SEQ ID NO: 9029	0.02 %	1.35
6	27	SEQ ID NO: 9030	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	38	SEQ ID NO: 9031	1.85 %	225
2	17	SEQ ID NO: 9032	0.02 %	3.6
3	2	SEQ ID NO: 9033	0.02 %	2.7
4	37	SEQ ID NO: 9034	0.01 %	1.8
5	27	SEQ ID NO: 9035	0.01 %	1.35
6	13	SEQ ID NO: 9036	0.00 %	0.675
7	14	SEQ ID NO: 9037	0.00 %	0.6

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	13	SEQ ID NO: 9038	0.04 %	6
2	37	SEQ ID NO: 9039	0.01 %	2.025

3	2	SEQ ID NO: 9040	0.00 %	0.9
4	19	SEQ ID NO: 9041	0.00 %	0.675
5	16	SEQ ID NO: 9042	0.00 %	0.54

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	20	SEQ ID NO: 9043	1.25 %	20
2	6	SEQ ID NO: 9044	0.52 %	8.4
3	5	SEQ ID NO: 9045	0.51 %	8.25
4	35	SEQ ID NO: 9046	0.36 %	5.76
5	31	SEQ ID NO: 9047	0.35 %	5.6
6	43	SEQ ID NO: 9048	0.27 %	4.4
7	13	SEQ ID NO: 9049	0.26 %	4.2
8	32	SEQ ID NO: 9050	0.21 %	3.36
9	2	SEQ ID NO: 9051	0.11 %	1.8
10	9	SEQ ID NO: 9052	0.10 %	1.68
11	8	SEQ ID NO: 9053	0.09 %	1.5
12	15	SEQ ID NO: 9054	0.09 %	1.5
13	23	SEQ ID NO: 9055	0.09 %	1.5
14	27	SEQ ID NO: 9056	0.08 %	1.4
15	24	SEQ ID NO: 9057	0.07 %	1.2
16	7	SEQ ID NO: 9058	0.06 %	1
17	17	SEQ ID NO: 9059	0.06 %	1
18	10	SEQ ID NO: 9060	0.05 %	0.9
19	39	SEQ ID NO: 9061	0.04 %	0.792
20	47	SEQ ID NO: 9062	0.04 %	0.792

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	5	SEQ ID NO: 9063	2.63 %	42
2	34	SEQ ID NO: 9064	0.54 %	8.64
3	30	SEQ ID NO: 9065	0.52 %	8.4
4	19	SEQ ID NO: 9066	0.50 %	8
5	50	SEQ ID NO: 9067	0.33 %	5.28
6	12	SEQ ID NO: 9068	0.26 %	4.2
7	31	SEQ ID NO: 9069	0.21 %	3.36
8	26	SEQ ID NO: 9070	0.15 %	2.52

9	8	SEQ ID NO: 9071	0.13 %	2.1
10	22	SEQ ID NO: 9072	0.12 %	2
11	23	SEQ ID NO: 9073	0.11 %	1.8
12	6	SEQ ID NO: 9074	0.09 %	1.5
13	14	SEQ ID NO: 9075	0.09 %	1.5
14	16	SEQ ID NO: 9076	0.09 %	1.5
15	7	SEQ ID NO: 9077	0.06 %	1
16	48	SEQ ID NO: 9078	0.04 %	0.75
17	0	SEQ ID NO: 9079	0.04 %	0.72
18	9	SEQ ID NO: 9080	0.04 %	0.72
19	47	SEQ ID NO: 9081	0.04 %	0.66
20	39	SEQ ID NO: 9082	0.03 %	0.6

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	15	SEQ ID NO: 9083	0.00 %	14.1442686
2	27	SEQ ID NO: 9084	0.00 %	9.598176
3	22	SEQ ID NO: 9085	0.00 %	9.5634
4	9	SEQ ID NO: 9086	0.00 %	5.546246013
5	2	SEQ ID NO: 9087	0.00 %	5.526462816
6	24	SEQ ID NO: 9088	0.00 %	4.88163753
7	17	SEQ ID NO: 9089	0.00 %	3.699285408
8	31	SEQ ID NO: 9090	0.00 %	2.29699206
9	6	SEQ ID NO: 9091	0.00 %	2.0016040674
10	7	SEQ ID NO: 9092	0.00 %	0.91287
11	49	SEQ ID NO: 9093	0.00 %	0.71805678
12	16	SEQ ID NO: 9094	0.00 %	0.6694257042
13	12	SEQ ID NO: 9095	0.00 %	0.6539828625

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	16	SEQ ID NO: 9096	0.00 %	34.28765802
2	19	SEQ ID NO: 9097	0.00 %	18.9368775
3	14	SEQ ID NO: 9098	0.00 %	14.1442686
4	27	SEQ ID NO: 9099	0.00 %	11.406528
5	26	SEQ ID NO: 9100	0.00 %	10.9304361558
6	34	SEQ ID NO: 9101	0.00 %	5.580927

7	6	SEQ ID NO: 9102	0.00 %	4.865742
8	9	SEQ ID NO: 9103	0.00 %	2.64106953
9	50	SEQ ID NO: 9104	0.00 %	2.6275752
10	30	SEQ ID NO: 9105	0.00 %	2.29699206
11	7	SEQ ID NO: 9106	0.00 %	0.86083641
12	42	SEQ ID NO: 9107	0.00 %	0.7049592
13	22	SEQ ID NO: 9108	0.00 %	0.6628440357
14	2	SEQ ID NO: 9109	0.00 %	0.6530644656

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	37	SEQ ID NO: 9110	15 %	5.4
2	38	SEQ ID NO: 9111	2.22 %	0.8

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	37	SEQ ID NO: 9112	7.5 %	2.7

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	35	SEQ ID NO: 9113	3.70 %	200
2	17	SEQ ID NO: 9114	0.11 %	6
3	6	SEQ ID NO: 9115	0.07 %	4
4	20	SEQ ID NO: 9116	0.07 %	4
5	31	SEQ ID NO: 9117	0.07 %	4
6	43	SEQ ID NO: 9118	0.07 %	4
7	7	SEQ ID NO: 9119	0.03 %	2
8	23	SEQ ID NO: 9120	0.02 %	1.2
9	24	SEQ ID NO: 9121	0.02 %	1.2
10	10	SEQ ID NO: 9122	0.01 %	0.9

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	35	SEQ ID NO: 9123	0.09 %	5
2	19	SEQ ID NO: 9124	0.07 %	4

3	30	SEQ ID NO: 9125	0.07 %	4
4	34	SEQ ID NO: 9126	0.07 %	4
5	7	SEQ ID NO: 9127	0.03 %	2
6	16	SEQ ID NO: 9128	0.03 %	1.8
7	23	SEQ ID NO: 9129	0.02 %	1.2
8	50	SEQ ID NO: 9130	0.02 %	1.2
9	9	SEQ ID NO: 9131	0.01 %	1

Table 22: Epitopes for SEQ ID NO: 6048

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	66	SEQ ID NO: 9132	0.44 %	25
2	80	SEQ ID NO: 9133	0.08 %	5
3	93	SEQ ID NO: 9134	0.04 %	2.7
4	11	SEQ ID NO: 9135	0.04 %	2.5
5	89	SEQ ID NO: 9136	0.04 %	2.25
6	48	SEQ ID NO: 9137	0.01 %	1
7	3	SEQ ID NO: 9138	0.00 %	0.5
8	9	SEQ ID NO: 9139	0.00 %	0.5
9	56	SEQ ID NO: 9140	0.00 %	0.5
10	101	SEQ ID NO: 9141	0.00 %	0.5
11	106	SEQ ID NO: 9142	0.00 %	0.5
12	110	SEQ ID NO: 9143	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	30	SEQ ID NO: 9144	0.4 %	22.5
2	88	SEQ ID NO: 9145	0.12 %	6.75
3	48	SEQ ID NO: 9146	0.04 %	2.5
4	55	SEQ ID NO: 9147	0.02 %	1.25
5	13	SEQ ID NO: 9148	0.01 %	0.9
6	79	SEQ ID NO: 9149	0.01 %	0.75
7	93	SEQ ID NO: 9150	0.01 %	0.675
8	2	SEQ ID NO: 9151	0.00 %	0.5
9	8	SEQ ID NO: 9152	0.00 %	0.5

10	65	SEQ ID NO: 9153	0.00 %	0.5
11	66	SEQ ID NO: 9154	0.00 %	0.5
12	80	SEQ ID NO: 9155	0.00 %	0.5
13	105	SEQ ID NO: 9156	0.00 %	0.5
14	109	SEQ ID NO: 9157	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	109	SEQ ID NO: 9158	0.74 %	90
2	3	SEQ ID NO: 9159	0.24 %	30
3	111	SEQ ID NO: 9160	0.12 %	15
4	106	SEQ ID NO: 9161	0.07 %	9
5	95	SEQ ID NO: 9162	0.05 %	6.075
6	101	SEQ ID NO: 9163	0.04 %	6
7	110	SEQ ID NO: 9164	0.02 %	3.6
8	84	SEQ ID NO: 9165	0.02 %	3
9	80	SEQ ID NO: 9166	0.02 %	2.7
10	37	SEQ ID NO: 9167	0.01 %	2.25
11	9	SEQ ID NO: 9168	0.01 %	2
12	54	SEQ ID NO: 9169	0.01 %	2
13	99	SEQ ID NO: 9170	0.01 %	1.35
14	1	SEQ ID NO: 9171	0.01 %	1.215
15	11	SEQ ID NO: 9172	0.00 %	0.9
16	15	SEQ ID NO: 9173	0.00 %	0.9
17	69	SEQ ID NO: 9174	0.00 %	0.6
18	5	SEQ ID NO: 9175	0.00 %	0.54
19	103	SEQ ID NO: 9176	0.00 %	0.54

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	75	SEQ ID NO: 9177	0.49 %	60
2	109	SEQ ID NO: 9178	0.29 %	36
3	22	SEQ ID NO: 9179	0.14 %	18
4	15	SEQ ID NO: 9180	0.04 %	6
5	110	SEQ ID NO: 9181	0.01 %	2.25
6	95	SEQ ID NO: 9182	0.01 %	1.8
7	101	SEQ ID NO: 9183	0.01 %	1.35

8	43	SEQ ID NO: 9184	0.00 %	1
9	2	SEQ ID NO: 9185	0.00 %	0.9
10	5	SEQ ID NO: 9186	0.00 %	0.9
11	7	SEQ ID NO: 9187	0.00 %	0.9
12	107	SEQ ID NO: 9188	0.00 %	0.9
13	102	SEQ ID NO: 9189	0.00 %	0.81
14	3	SEQ ID NO: 9190	0.00 %	0.75
15	8	SEQ ID NO: 9191	0.00 %	0.6
16	103	SEQ ID NO: 9192	0.00 %	0.54

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	88	SEQ ID NO: 9193	1.66 %	26.6112
2	77	SEQ ID NO: 9194	0.77 %	12.32
3	18	SEQ ID NO: 9195	0.56 %	9
4	108	SEQ ID NO: 9196	0.56 %	9
5	92	SEQ ID NO: 9197	0.54 %	8.64
6	96	SEQ ID NO: 9198	0.54 %	8.64
7	73	SEQ ID NO: 9199	0.46 %	7.5
8	40	SEQ ID NO: 9200	0.45 %	7.2
9	104	SEQ ID NO: 9201	0.42 %	6.72
10	8	SEQ ID NO: 9202	0.41 %	6.6
11	21	SEQ ID NO: 9203	0.37 %	6
12	102	SEQ ID NO: 9204	0.37 %	6
13	22	SEQ ID NO: 9205	0.25 %	4
14	68	SEQ ID NO: 9206	0.25 %	4
15	106	SEQ ID NO: 9207	0.22 %	3.6
16	1	SEQ ID NO: 9208	0.18 %	3
17	79	SEQ ID NO: 9209	0.18 %	3
18	93	SEQ ID NO: 9210	0.18 %	3
19	101	SEQ ID NO: 9211	0.18 %	3
20	37	SEQ ID NO: 9212	0.15 %	2.4

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	100	SEQ ID NO: 9213	0.93 %	15
2	18	SEQ ID NO: 9214	0.78 %	12.6

3	98	SEQ ID NO: 9215	0.52 %	8.4
4	73	SEQ ID NO: 9216	0.46 %	7.5
5	91	SEQ ID NO: 9217	0.45 %	7.2
6	103	SEQ ID NO: 9218	0.42 %	6.72
7	7	SEQ ID NO: 9219	0.41 %	6.6
8	21	SEQ ID NO: 9220	0.37 %	6
9	46	SEQ ID NO: 9221	0.37 %	6
10	93	SEQ ID NO: 9222	0.37 %	6
11	96	SEQ ID NO: 9223	0.37 %	6
12	101	SEQ ID NO: 9224	0.37 %	6
13	77	SEQ ID NO: 9225	0.25 %	4
14	92	SEQ ID NO: 9226	0.22 %	3.6
15	105	SEQ ID NO: 9227	0.22 %	3.6
16	2	SEQ ID NO: 9228	0.18 %	3
17	53	SEQ ID NO: 9229	0.18 %	3
18	36	SEQ ID NO: 9230	0.12 %	2
19	55	SEQ ID NO: 9231	0.12 %	2
20	102	SEQ ID NO: 9232	0.11 %	1.8

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	84	SEQ ID NO: 9233	0.01 %	441.342216
2	102	SEQ ID NO: 9234	0.00 %	63.16728165
3	107	SEQ ID NO: 9235	0.00 %	51.882640425
4	1	SEQ ID NO: 9236	0.00 %	43.8816609
5	95	SEQ ID NO: 9237	0.00 %	33.40165248
6	2	SEQ ID NO: 9238	0.00 %	24.66305226
7	92	SEQ ID NO: 9239	0.00 %	22.64458905
8	103	SEQ ID NO: 9240	0.00 %	20.70206586
9	47	SEQ ID NO: 9241	0.00 %	11.175953184
10	94	SEQ ID NO: 9242	0.00 %	8.452983
11	15	SEQ ID NO: 9243	0.00 %	8.1793152
12	8	SEQ ID NO: 9244	0.00 %	4.993461
13	5	SEQ ID NO: 9245	0.00 %	4.57284528
14	99	SEQ ID NO: 9246	0.00 %	3.999468528
15	105	SEQ ID NO: 9247	0.00 %	2.231322
16	20	SEQ ID NO: 9248	0.00 %	1.3524
17	62	SEQ ID NO: 9249	0.00 %	0.8631693

18	6	SEQ ID NO: 9250	0.00 %	0.824619
19	57	SEQ ID NO: 9251	0.00 %	0.72105
20	58	SEQ ID NO: 9252	0.00 %	0.7147572

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	101	SEQ ID NO: 9253	0.03 %	1243.078056
2	3	SEQ ID NO: 9254	0.01 %	592.944462
3	106	SEQ ID NO: 9255	0.00 %	94.2678
4	5	SEQ ID NO: 9256	0.00 %	43.42032
5	107	SEQ ID NO: 9257	0.00 %	33.30332334
6	102	SEQ ID NO: 9258	0.00 %	32.53181778
7	54	SEQ ID NO: 9259	0.00 %	27.324
8	7	SEQ ID NO: 9260	0.00 %	21.3624
9	1	SEQ ID NO: 9261	0.00 %	13.723479
10	95	SEQ ID NO: 9262	0.00 %	13.00344192
11	94	SEQ ID NO: 9263	0.00 %	10.01276388
12	99	SEQ ID NO: 9264	0.00 %	5.6615328
13	39	SEQ ID NO: 9265	0.00 %	3.6304212
14	111	SEQ ID NO: 9266	0.00 %	2.53368
15	103	SEQ ID NO: 9267	0.00 %	2.475394803
16	14	SEQ ID NO: 9268	0.00 %	2.4519012
17	19	SEQ ID NO: 9269	0.00 %	2.07604992
18	29	SEQ ID NO: 9270	0.00 %	1.8179154
19	57	SEQ ID NO: 9271	0.00 %	1.52076
20	47	SEQ ID NO: 9272	0.00 %	1.27712376

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	80	SEQ ID NO: 9273	3.33 %	1.2
2	69	SEQ ID NO: 9274	1.66 %	0.6
3	109	SEQ ID NO: 9275	1.66 %	0.6

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	22	SEQ ID NO: 9276	11.11 %	4

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	Start position	Sequence	% of max. score	Score
1	22	SEQ ID NO: 9277	3.70 %	200
2	77	SEQ ID NO: 9278	2.22 %	120
3	104	SEQ ID NO: 9279	0.22 %	12
4	40	SEQ ID NO: 9280	0.11 %	6
5	8	SEQ ID NO: 9281	0.07 %	4
6	21	SEQ ID NO: 9282	0.07 %	4
7	68	SEQ ID NO: 9283	0.07 %	4
8	92	SEQ ID NO: 9284	0.07 %	4
9	102	SEQ ID NO: 9285	0.07 %	4
10	46	SEQ ID NO: 9286	0.03 %	2
11	98	SEQ ID NO: 9287	0.03 %	2
12	103	SEQ ID NO: 9288	0.03 %	2
13	88	SEQ ID NO: 9289	0.02 %	1.2
14	105	SEQ ID NO: 9290	0.01 %	0.9
15	43	SEQ ID NO: 9291	0.01 %	0.6
16	79	SEQ ID NO: 9292	0.01 %	0.6
17	95	SEQ ID NO: 9293	0.01 %	0.6
18	107	SEQ ID NO: 9294	0.00 %	0.5

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	Start position	Sequence	% of max. score	Score
1	46	SEQ ID NO: 9295	1.48 %	80
2	98	SEQ ID NO: 9296	1.48 %	80
3	91	SEQ ID NO: 9297	0.37 %	20
4	103	SEQ ID NO: 9298	0.37 %	20
5	7	SEQ ID NO: 9299	0.07 %	4
6	21	SEQ ID NO: 9300	0.07 %	4
7	101	SEQ ID NO: 9301	0.07 %	4
8	107	SEQ ID NO: 9302	0.03 %	2
9	67	SEQ ID NO: 9303	0.02 %	1.2
10	93	SEQ ID NO: 9304	0.02 %	1.2
11	69	SEQ ID NO: 9305	0.01 %	1
12	39	SEQ ID NO: 9306	0.01 %	0.6
13	77	SEQ ID NO: 9307	0.01 %	0.6

14	22	SEQ ID NO: 9308	0.00 %	0.5

Table 23: Epitopes for SEQ ID NO: 6049

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	0	SEQ ID NO: 9309	0.2 %	11.25
2	35	SEQ ID NO: 9310	0.01 %	0.9
3	4	SEQ ID NO: 9311	0.00 %	0.5
4	5	SEQ ID NO: 9312	0.00 %	0.5
5	10	SEQ ID NO: 9313	0.00 %	0.5
6	19	SEQ ID NO: 9314	0.00 %	0.5
7	21	SEQ ID NO: 9315	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	0	SEQ ID NO: 9316	0.2 %	11.25
2	5	SEQ ID NO: 9317	0.04 %	2.5
3	33	SEQ ID NO: 9318	0.02 %	1.5
4	3	SEQ ID NO: 9319	0.02 %	1.25
5	9	SEQ ID NO: 9320	0.00 %	0.5
6	18	SEQ ID NO: 9321	0.00 %	0.5
7	20	SEQ ID NO: 9322	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	4	SEQ ID NO: 9323	0.14 %	18
2	16	SEQ ID NO: 9324	0.11 %	13.5
3	23	SEQ ID NO: 9325	0.06 %	8.1
4	18	SEQ ID NO: 9326	0.03 %	4.05
5	21	SEQ ID NO: 9327	0.01 %	2.025
6	9	SEQ ID NO: 9328	0.01 %	1.8
7	15	SEQ ID NO: 9329	0.01 %	1.8
8	25	SEQ ID NO: 9330	0.01 %	1.8
9	12	SEQ ID NO: 9331	0.00 %	0.9

10	19	SEQ ID NO: 9332	0.00 %	0.9
11	20	SEQ ID NO: 9333	0.00 %	0.9
12	2	SEQ ID NO: 9334	0.00 %	0.81
13	22	SEQ ID NO: 9335	0.00 %	0.81
14	10	SEQ ID NO: 9336	0.00 %	0.6

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	20	SEQ ID NO: 9337	0.16 %	20.25
2	9	SEQ ID NO: 9338	0.09 %	12
3	16	SEQ ID NO: 9339	0.07 %	9
4	18	SEQ ID NO: 9340	0.07 %	9
5	22	SEQ ID NO: 9341	0.06 %	8.1
6	4	SEQ ID NO: 9342	0.03 %	4.05
7	15	SEQ ID NO: 9343	0.03 %	4.05
8	12	SEQ ID NO: 9344	0.02 %	3.6
9	3	SEQ ID NO: 9345	0.00 %	0.9
10	33	SEQ ID NO: 9346	0.00 %	0.6
11	2	SEQ ID NO: 9347	0.00 %	0.54
12	24	SEQ ID NO: 9348	0.00 %	0.54

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	8	SEQ ID NO: 9349	18.78 %	300
2	11	SEQ ID NO: 9350	1.87 %	30
3	28	SEQ ID NO: 9351	1.50 %	24
4	7	SEQ ID NO: 9352	0.75 %	12
5	17	SEQ ID NO: 9353	0.56 %	9
6	14	SEQ ID NO: 9354	0.46 %	7.5
7	23	SEQ ID NO: 9355	0.37 %	6
8	13	SEQ ID NO: 9356	0.36 %	5.76
9	2	SEQ ID NO: 9357	0.35 %	5.6
10	16	SEQ ID NO: 9358	0.35 %	5.6
11	9	SEQ ID NO: 9359	0.30 %	4.8
12	21	SEQ ID NO: 9360	0.26 %	4.2
13	5	SEQ ID NO: 9361	0.25 %	4
14	4	SEQ ID NO: 9362	0.22 %	3.6

15	0	SEQ ID NO: 9363	0.18 %	3
16	19	SEQ ID NO: 9364	0.18 %	3
17	10	SEQ ID NO: 9365	0.15 %	2.4
18	18	SEQ ID NO: 9366	0.13 %	2.1
19	25	SEQ ID NO: 9367	0.06 %	1.1
20	15	SEQ ID NO: 9368	0.05 %	0.9

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	8	SEQ ID NO: 9369	22.54 %	360
2	7	SEQ ID NO: 9370	1.25 %	20
3	17	SEQ ID NO: 9371	0.65 %	10.5
4	15	SEQ ID NO: 9372	0.52 %	8.4
5	4	SEQ ID NO: 9373	0.45 %	7.2
6	22	SEQ ID NO: 9374	0.37 %	6
7	12	SEQ ID NO: 9375	0.36 %	5.76
8	27	SEQ ID NO: 9376	0.30 %	4.8
9	14	SEQ ID NO: 9377	0.28 %	4.5
10	20	SEQ ID NO: 9378	0.26 %	4.2
11	10	SEQ ID NO: 9379	0.25 %	4
12	3	SEQ ID NO: 9380	0.18 %	3
13	18	SEQ ID NO: 9381	0.18 %	3
14	9	SEQ ID NO: 9382	0.15 %	2.4
15	24	SEQ ID NO: 9383	0.10 %	1.65
16	16	SEQ ID NO: 9384	0.07 %	1.2
17	13	SEQ ID NO: 9385	0.06 %	1
18	11	SEQ ID NO: 9386	0.05 %	0.9
19	1	SEQ ID NO: 9387	0.05 %	0.84

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	12	SEQ ID NO: 9388	0.10 %	4267.988928
2	23	SEQ ID NO: 9389	0.03 %	1360.69088544
3	9	SEQ ID NO: 9390	0.01 %	569.948832
4	16	SEQ ID NO: 9391	0.00 %	309.0498408
5	15	SEQ ID NO: 9392	0.00 %	79.73570448
6	2	SEQ ID NO: 9393	0.00 %	51.109542

7	18	SEQ ID NO: 9394	0.00 %	45.25539984
8	25	SEQ ID NO: 9395	0.00 %	34.28765802
9	22	SEQ ID NO: 9396	0.00 %	26.532116325
10	5	SEQ ID NO: 9397	0.00 %	25.26691266
11	21	SEQ ID NO: 9398	0.00 %	4.72873208445
12	11	SEQ ID NO: 9399	0.00 %	2.638538265
13	8	SEQ ID NO: 9400	0.00 %	2.4274552038
14	4	SEQ ID NO: 9401	0.00 %	1.7415324
15	20	SEQ ID NO: 9402	0.00 %	1.6025526
16	13	SEQ ID NO: 9403	0.00 %	1.453803297
17	35	SEQ ID NO: 9404	0.00 %	1.36878336
18	3	SEQ ID NO: 9405	0.00 %	0.824619
19	33	SEQ ID NO: 9406	0.00 %	0.513774

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	22	SEQ ID NO: 9407	0.09 %	3636.068421648
2	4	SEQ ID NO: 9408	0.02 %	1107.960876
3	15	SEQ ID NO: 9409	0.02 %	836.2525104
4	16	SEQ ID NO: 9410	0.00 %	150.9313176
5	12	SEQ ID NO: 9411	0.00 %	76.55002416
6	1	SEQ ID NO: 9412	0.00 %	49.0273014
7	10	SEQ ID NO: 9413	0.00 %	42.1638414747
8	20	SEQ ID NO: 9414	0.00 %	9.29480508
9	24	SEQ ID NO: 9415	0.00 %	9.2669346
10	13	SEQ ID NO: 9416	0.00 %	7.96581954
11	21	SEQ ID NO: 9417	0.00 %	5.051306761875
12	5	SEQ ID NO: 9418	0.00 %	2.6941464
13	11	SEQ ID NO: 9419	0.00 %	2.3839914
14	34	SEQ ID NO: 9420	0.00 %	1.465422
15	2	SEQ ID NO: 9421	0.00 %	0.70794
16	9	SEQ ID NO: 9422	0.00 %	0.6513048
17	19	SEQ ID NO: 9423	0.00 %	0.51882640425

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	33	SEQ ID NO: 9424	1.66 %	0.6

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	13	SEQ ID NO: 9425	0.22 %	12
2	2	SEQ ID NO: 9426	0.07 %	4
3	9	SEQ ID NO: 9427	0.07 %	4
4	16	SEQ ID NO: 9428	0.07 %	4
5	23	SEQ ID NO: 9429	0.07 %	4
6	5	SEQ ID NO: 9430	0.02 %	1.2
7	15	SEQ ID NO: 9431	0.01 %	1

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	4	SEQ ID NO: 9432	0.07 %	4
2	10	SEQ ID NO: 9433	0.07 %	4
3	12	SEQ ID NO: 9434	0.07 %	4
4	15	SEQ ID NO: 9435	0.07 %	4
5	22	SEQ ID NO: 9436	0.07 %	4
6	13	SEQ ID NO: 9437	0.02 %	1.2

Table 24: Epitopes for SEQ ID NO: 6050

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	47	SEQ ID NO: 9438	0.01 %	0.75
2	21	SEQ ID NO: 9439	0.00 %	0.5
3	53	SEQ ID NO: 9440	0.00 %	0.5

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**

1	16	SEQ ID NO: 9441	0.04 %	2.5
2	71	SEQ ID NO: 9442	0.04 %	2.5
3	47	SEQ ID NO: 9443	0.02 %	1.5
4	62	SEQ ID NO: 9444	0.01 %	0.9
5	20	SEQ ID NO: 9445	0.00 %	0.5
6	38	SEQ ID NO: 9446	0.00 %	0.5

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	54	SEQ ID NO: 9447	0.02 %	2.7
2	17	SEQ ID NO: 9448	0.01 %	2
3	3	SEQ ID NO: 9449	0.01 %	1.8

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	22	SEQ ID NO: 9450	0.09 %	12
2	16	SEQ ID NO: 9451	0.01 %	2
3	54	SEQ ID NO: 9452	0.00 %	0.9

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	70	SEQ ID NO: 9453	2.10 %	33.6
2	7	SEQ ID NO: 9454	1.12 %	18
3	60	SEQ ID NO: 9455	0.46 %	7.5
4	54	SEQ ID NO: 9456	0.37 %	6
5	14	SEQ ID NO: 9457	0.31 %	5
6	19	SEQ ID NO: 9458	0.30 %	4.8
7	47	SEQ ID NO: 9459	0.30 %	4.8
8	12	SEQ ID NO: 9460	0.25 %	4
9	15	SEQ ID NO: 9461	0.25 %	4
10	67	SEQ ID NO: 9462	0.25 %	4
11	21	SEQ ID NO: 9463	0.18 %	3
12	37	SEQ ID NO: 9464	0.06 %	1
13	27	SEQ ID NO: 9465	0.03 %	0.5

HLA A24 - 10 mers

Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	14	SEQ ID NO: 9466	12.52 %	200
2	7	SEQ ID NO: 9467	0.93 %	15
3	11	SEQ ID NO: 9468	0.75 %	12
4	60	SEQ ID NO: 9469	0.56 %	9
5	18	SEQ ID NO: 9470	0.45 %	7.2
6	46	SEQ ID NO: 9471	0.45 %	7.2
7	53	SEQ ID NO: 9472	0.37 %	6
8	69	SEQ ID NO: 9473	0.35 %	5.6
9	66	SEQ ID NO: 9474	0.25 %	4
10	20	SEQ ID NO: 9475	0.12 %	2
11	47	SEQ ID NO: 9476	0.07 %	1.2
12	36	SEQ ID NO: 9477	0.06 %	1
13	26	SEQ ID NO: 9478	0.04 %	0.75
14	70	SEQ ID NO: 9479	0.04 %	0.72

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	54	SEQ ID NO: 9480	0.02 %	881.199
2	26	SEQ ID NO: 9481	0.00 %	95.013
3	61	SEQ ID NO: 9482	0.00 %	93.69648
4	19	SEQ ID NO: 9483	0.00 %	40.2894864
5	74	SEQ ID NO: 9484	0.00 %	12.6684
6	35	SEQ ID NO: 9485	0.00 %	10.34586
7	69	SEQ ID NO: 9486	0.00 %	3.3704706
8	13	SEQ ID NO: 9487	0.00 %	1.656
9	15	SEQ ID NO: 9488	0.00 %	1.47537042
10	68	SEQ ID NO: 9489	0.00 %	0.966
11	22	SEQ ID NO: 9490	0.00 %	0.942678
12	12	SEQ ID NO: 9491	0.00 %	0.7669695
13	36	SEQ ID NO: 9492	0.00 %	0.52661835

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	61	SEQ ID NO: 9493	0.00 %	93.69648
2	25	SEQ ID NO: 9494	0.00 %	63.33035625

3	34	SEQ ID NO: 9495	0.00 %	50.232
4	53	SEQ ID NO: 9496	0.00 %	45.2838375
5	26	SEQ ID NO: 9497	0.00 %	14.35752
6	27	SEQ ID NO: 9498	0.00 %	2.8557858
7	17	SEQ ID NO: 9499	0.00 %	2.3973222
8	36	SEQ ID NO: 9500	0.00 %	1.798209
9	69	SEQ ID NO: 9501	0.00 %	1.03521597
10	67	SEQ ID NO: 9502	0.00 %	0.966
11	68	SEQ ID NO: 9503	0.00 %	0.910938
12	11	SEQ ID NO: 9504	0.00 %	0.7669695

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	17	SEQ ID NO: 9505	2.22 %	0.8

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	16	SEQ ID NO: 9506	5.55 %	2

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	27	SEQ ID NO: 9507	0.37 %	20
2	54	SEQ ID NO: 9508	0.22 %	12
3	70	SEQ ID NO: 9509	0.22 %	12
4	67	SEQ ID NO: 9510	0.11 %	6
5	12	SEQ ID NO: 9511	0.07 %	4
6	15	SEQ ID NO: 9512	0.07 %	4
7	19	SEQ ID NO: 9513	0.07 %	4
8	49	SEQ ID NO: 9514	0.03 %	2
9	69	SEQ ID NO: 9515	0.03 %	1.8
10	47	SEQ ID NO: 9516	0.02 %	1.2
11	5	SEQ ID NO: 9517	0.01 %	1
12	9	SEQ ID NO: 9518	0.01 %	1
13	35	SEQ ID NO: 9519	0.01 %	1
14	37	SEQ ID NO: 9520	0.01 %	0.6
15	68	SEQ ID NO: 9521	0.01 %	0.6

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	Start position	Sequence	% of max. score	Score
1	69	SEQ ID NO: 9522	0.66 %	36
2	53	SEQ ID NO: 9523	0.22 %	12
3	5	SEQ ID NO: 9524	0.13 %	7.5
4	66	SEQ ID NO: 9525	0.11 %	6
5	11	SEQ ID NO: 9526	0.07 %	4
6	27	SEQ ID NO: 9527	0.07 %	4
7	46	SEQ ID NO: 9528	0.07 %	4
8	18	SEQ ID NO: 9529	0.02 %	1.2
9	9	SEQ ID NO: 9530	0.01 %	1
10	26	SEQ ID NO: 9531	0.01 %	1
11	25	SEQ ID NO: 9532	0.01 %	0.75
12	17	SEQ ID NO: 9533	0.01 %	0.6
13	36	SEQ ID NO: 9534	0.01 %	0.6
14	68	SEQ ID NO: 9535	0.01 %	0.6
15	35	SEQ ID NO: 9536	0.00 %	0.5
16	42	SEQ ID NO: 9537	0.00 %	0.5
17	73	SEQ ID NO: 9538	0.00 %	0.5

Table 25: Epitopes for SEQ ID NO: 6052

HLA A1 - 9 mers				
Maximum possible score using this molecule type				5625
Rank	Start position	Sequence	% of max. score	Score
1	365	SEQ ID NO: 9539	0.8 %	45
2	397	SEQ ID NO: 9540	0.44 %	25
3	229	SEQ ID NO: 9541	0.32 %	18
4	103	SEQ ID NO: 9542	0.17 %	10
5	338	SEQ ID NO: 9543	0.17 %	10
6	251	SEQ ID NO: 9544	0.16 %	9
7	79	SEQ ID NO: 9545	0.11 %	6.25
8	119	SEQ ID NO: 9546	0.10 %	6
9	361	SEQ ID NO: 9547	0.08 %	5
10	60	SEQ ID NO: 9548	0.04 %	2.25
11	101	SEQ ID NO: 9549	0.04 %	2.25
12	278	SEQ ID NO: 9550	0.04 %	2.25

13	23	SEQ ID NO: 9551	0.02 %	1.25
14	164	SEQ ID NO: 9552	0.02 %	1.25
15	165	SEQ ID NO: 9553	0.02 %	1.25
16	295	SEQ ID NO: 9554	0.02 %	1.25
17	172	SEQ ID NO: 9555	0.01 %	0.9
18	0	SEQ ID NO: 9556	0.01 %	0.75
19	311	SEQ ID NO: 9557	0.01 %	0.75
20	78	SEQ ID NO: 9558	0.01 %	0.625

HLA A1 - 10 mers				
Maximum possible score using this molecule type				5625
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	114	SEQ ID NO: 9559	1.11 %	62.5
2	134	SEQ ID NO: 9560	0.8 %	45
3	365	SEQ ID NO: 9561	0.8 %	45
4	77	SEQ ID NO: 9562	0.66 %	37.5
5	103	SEQ ID NO: 9563	0.44 %	25
6	23	SEQ ID NO: 9564	0.22 %	12.5
7	338	SEQ ID NO: 9565	0.17 %	10
8	361	SEQ ID NO: 9566	0.17 %	10
9	324	SEQ ID NO: 9567	0.11 %	6.25
10	375	SEQ ID NO: 9568	0.11 %	6.25
11	79	SEQ ID NO: 9569	0.04 %	2.5
12	295	SEQ ID NO: 9570	0.04 %	2.5
13	346	SEQ ID NO: 9571	0.04 %	2.5
14	378	SEQ ID NO: 9572	0.03 %	2
15	251	SEQ ID NO: 9573	0.03 %	1.8
16	214	SEQ ID NO: 9574	0.02 %	1.125
17	160	SEQ ID NO: 9575	0.01 %	1
18	172	SEQ ID NO: 9576	0.01 %	0.9
19	229	SEQ ID NO: 9577	0.01 %	0.9
20	376	SEQ ID NO: 9578	0.01 %	0.9

HLA A3 - 9 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	229	SEQ ID NO: 9579	0.49 %	60
2	361	SEQ ID NO: 9580	0.27 %	33.75
3	330	SEQ ID NO: 9581	0.16 %	20

4	218	SEQ ID NO: 9582	0.09 %	12
5	338	SEQ ID NO: 9583	0.04 %	6
6	352	SEQ ID NO: 9584	0.04 %	6
7	103	SEQ ID NO: 9585	0.04 %	5.4
8	291	SEQ ID NO: 9586	0.01 %	2
9	241	SEQ ID NO: 9587	0.01 %	1.8
10	290	SEQ ID NO: 9588	0.01 %	1.8
11	316	SEQ ID NO: 9589	0.01 %	1.8
12	222	SEQ ID NO: 9590	0.01 %	1.35
13	266	SEQ ID NO: 9591	0.01 %	1.35
14	53	SEQ ID NO: 9592	0.00 %	1
15	100	SEQ ID NO: 9593	0.00 %	0.9
16	138	SEQ ID NO: 9594	0.00 %	0.9
17	240	SEQ ID NO: 9595	0.00 %	0.9
18	119	SEQ ID NO: 9596	0.00 %	0.675
19	44	SEQ ID NO: 9597	0.00 %	0.6
20	161	SEQ ID NO: 9598	0.00 %	0.6

HLA A3 - 10 mers				
Maximum possible score using this molecule type				12150
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	338	SEQ ID NO: 9599	0.49 %	60
2	160	SEQ ID NO: 9600	0.32 %	40
3	352	SEQ ID NO: 9601	0.24 %	30
4	361	SEQ ID NO: 9602	0.18 %	22.5
5	103	SEQ ID NO: 9603	0.13 %	16.2
6	290	SEQ ID NO: 9604	0.07 %	9
7	351	SEQ ID NO: 9605	0.07 %	9
8	44	SEQ ID NO: 9606	0.04 %	6
9	228	SEQ ID NO: 9607	0.03 %	4.05
10	394	SEQ ID NO: 9608	0.02 %	3
11	240	SEQ ID NO: 9609	0.02 %	2.7
12	100	SEQ ID NO: 9610	0.01 %	1.8
13	114	SEQ ID NO: 9611	0.01 %	1.8
14	93	SEQ ID NO: 9612	0.01 %	1.5
15	134	SEQ ID NO: 9613	0.01 %	1.5
16	221	SEQ ID NO: 9614	0.01 %	1.35
17	330	SEQ ID NO: 9615	0.00 %	1.2
18	112	SEQ ID NO: 9616	0.00 %	0.9

19	218	SEQ ID NO: 9617	0.00 %	0.9
20	55	SEQ ID NO: 9618	0.00 %	0.6

HLA A24 - 9 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	345	SEQ ID NO: 9619	1.50 %	24
2	306	SEQ ID NO: 9620	0.75 %	12
3	222	SEQ ID NO: 9621	0.54 %	8.64
4	111	SEQ ID NO: 9622	0.51 %	8.25
5	159	SEQ ID NO: 9623	0.45 %	7.2
6	219	SEQ ID NO: 9624	0.45 %	7.2
7	283	SEQ ID NO: 9625	0.45 %	7.2
8	266	SEQ ID NO: 9626	0.42 %	6.72
9	56	SEQ ID NO: 9627	0.41 %	6.6
10	131	SEQ ID NO: 9628	0.37 %	6
11	214	SEQ ID NO: 9629	0.37 %	6
12	297	SEQ ID NO: 9630	0.37 %	6
13	86	SEQ ID NO: 9631	0.31 %	5
14	122	SEQ ID NO: 9632	0.31 %	5
15	48	SEQ ID NO: 9633	0.30 %	4.8
16	105	SEQ ID NO: 9634	0.30 %	4.8
17	213	SEQ ID NO: 9635	0.30 %	4.8
18	323	SEQ ID NO: 9636	0.30 %	4.8
19	338	SEQ ID NO: 9637	0.30 %	4.8
20	399	SEQ ID NO: 9638	0.30 %	4.8

HLA A24 - 10 mers				
Maximum possible score using this molecule type				1596.672
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	65	SEQ ID NO: 9639	0.93 %	15
2	306	SEQ ID NO: 9640	0.75 %	12
3	95	SEQ ID NO: 9641	0.66 %	10.56
4	36	SEQ ID NO: 9642	0.60 %	9.6
5	385	SEQ ID NO: 9643	0.50 %	8
6	111	SEQ ID NO: 9644	0.46 %	7.5
7	104	SEQ ID NO: 9645	0.45 %	7.2
8	214	SEQ ID NO: 9646	0.45 %	7.2
9	221	SEQ ID NO: 9647	0.45 %	7.2

10	277	SEQ ID NO: 9648	0.45 %	7.2
11	150	SEQ ID NO: 9649	0.37 %	6
12	152	SEQ ID NO: 9650	0.37 %	6
13	158	SEQ ID NO: 9651	0.37 %	6
14	171	SEQ ID NO: 9652	0.37 %	6
15	343	SEQ ID NO: 9653	0.37 %	6
16	110	SEQ ID NO: 9654	0.34 %	5.5
17	85	SEQ ID NO: 9655	0.31 %	5
18	47	SEQ ID NO: 9656	0.30 %	4.8
19	213	SEQ ID NO: 9657	0.30 %	4.8
20	218	SEQ ID NO: 9658	0.30 %	4.8

HLA A 0201 - 9 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	222	SEQ ID NO: 9659	0.03 %	1267.10434728
2	226	SEQ ID NO: 9660	0.00 %	69.552
3	316	SEQ ID NO: 9661	0.00 %	50.232
4	351	SEQ ID NO: 9662	0.00 %	31.24872
5	159	SEQ ID NO: 9663	0.00 %	13.6235739
6	406	SEQ ID NO: 9664	0.00 %	11.4264
7	165	SEQ ID NO: 9665	0.00 %	8.14407
8	238	SEQ ID NO: 9666	0.00 %	7.0518
9	138	SEQ ID NO: 9667	0.00 %	5.112072
10	130	SEQ ID NO: 9668	0.00 %	3.00547233
11	303	SEQ ID NO: 9669	0.00 %	2.59578
12	157	SEQ ID NO: 9670	0.00 %	2.412585
13	219	SEQ ID NO: 9671	0.00 %	2.103255861
14	305	SEQ ID NO: 9672	0.00 %	1.86369
15	158	SEQ ID NO: 9673	0.00 %	1.646892
16	331	SEQ ID NO: 9674	0.00 %	1.614048
17	399	SEQ ID NO: 9675	0.00 %	1.442246832
18	324	SEQ ID NO: 9676	0.00 %	1.319625
19	312	SEQ ID NO: 9677	0.00 %	1.233099
20	262	SEQ ID NO: 9678	0.00 %	0.966

HLA A 0201 - 10 mers				
Maximum possible score using this molecule type				3925227.1
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**

1	221	SEQ ID NO: 9679	0.00 %	309.0498408
2	112	SEQ ID NO: 9680	0.00 %	98.26704
3	330	SEQ ID NO: 9681	0.00 %	98.26704
4	158	SEQ ID NO: 9682	0.00 %	36.31608
5	218	SEQ ID NO: 9683	0.00 %	24.0754248
6	124	SEQ ID NO: 9684	0.00 %	12.2199
7	55	SEQ ID NO: 9685	0.00 %	10.467576
8	315	SEQ ID NO: 9686	0.00 %	7.7274204
9	350	SEQ ID NO: 9687	0.00 %	4.296699
10	405	SEQ ID NO: 9688	0.00 %	4.286487
11	388	SEQ ID NO: 9689	0.00 %	4.054785
12	322	SEQ ID NO: 9690	0.00 %	3.883803
13	130	SEQ ID NO: 9691	0.00 %	3.428691903
14	45	SEQ ID NO: 9692	0.00 %	3.411230625
15	132	SEQ ID NO: 9693	0.00 %	2.99943
16	410	SEQ ID NO: 9694	0.00 %	2.63718
17	316	SEQ ID NO: 9695	0.00 %	2.48686074
18	104	SEQ ID NO: 9696	0.00 %	2.477311485
19	164	SEQ ID NO: 9697	0.00 %	2.2011
20	282	SEQ ID NO: 9698	0.00 %	2.16591

HLA A 1101 - 9 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	361	SEQ ID NO: 9699	16.66 %	6
2	53	SEQ ID NO: 9700	2.77 %	1
3	240	SEQ ID NO: 9701	1.66 %	0.6
4	241	SEQ ID NO: 9702	1.66 %	0.6

HLA A 1101 - 10 mers				
Maximum possible score using this molecule type				36
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	361	SEQ ID NO: 9703	16.66 %	6
2	93	SEQ ID NO: 9704	8.33 %	3
3	338	SEQ ID NO: 9705	3.33 %	1.2
4	134	SEQ ID NO: 9706	2.77 %	1
5	228	SEQ ID NO: 9707	2.5 %	0.9
6	160	SEQ ID NO: 9708	2.22 %	0.8
7	239	SEQ ID NO: 9709	1.66 %	0.6

8	240	SEQ ID NO: 9710	1.66 %	0.6
9	257	SEQ ID NO: 9711	1.66 %	0.6
10	379	SEQ ID NO: 9712	1.66 %	0.6

HLA B7 - 9 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	105	SEQ ID NO: 9713	14.81 %	800
2	66	SEQ ID NO: 9714	1.48 %	80
3	93	SEQ ID NO: 9715	0.92 %	50
4	257	SEQ ID NO: 9716	0.55 %	30
5	323	SEQ ID NO: 9717	0.37 %	20
6	211	SEQ ID NO: 9718	0.22 %	12
7	219	SEQ ID NO: 9719	0.22 %	12
8	403	SEQ ID NO: 9720	0.18 %	10
9	343	SEQ ID NO: 9721	0.14 %	8
10	12	SEQ ID NO: 9722	0.11 %	6
11	113	SEQ ID NO: 9723	0.11 %	6
12	48	SEQ ID NO: 9724	0.07 %	4
13	56	SEQ ID NO: 9725	0.07 %	4
14	150	SEQ ID NO: 9726	0.07 %	4
15	153	SEQ ID NO: 9727	0.07 %	4
16	159	SEQ ID NO: 9728	0.07 %	4
17	213	SEQ ID NO: 9729	0.07 %	4
18	216	SEQ ID NO: 9730	0.07 %	4
19	222	SEQ ID NO: 9731	0.07 %	4
20	283	SEQ ID NO: 9732	0.07 %	4

HLA B7 - 10 mers				
Maximum possible score using this molecule type				5400
Rank	**Start position**	**Sequence**	**% of max. score**	**Score**
1	36	SEQ ID NO: 9733	1.48 %	80
2	150	SEQ ID NO: 9734	1.48 %	80
3	343	SEQ ID NO: 9735	1.48 %	80
4	12	SEQ ID NO: 9736	1.11 %	60
5	308	SEQ ID NO: 9737	1.11 %	60
6	130	SEQ ID NO: 9738	0.37 %	20
7	55	SEQ ID NO: 9739	0.22 %	12
8	210	SEQ ID NO: 9740	0.22 %	12

9	218	SEQ ID NO: 9741	0.22 %	12
10	201	SEQ ID NO: 9742	0.18 %	10
11	121	SEQ ID NO: 9743	0.14 %	8
12	391	SEQ ID NO: 9744	0.13 %	7.5
13	112	SEQ ID NO: 9745	0.11 %	6
14	385	SEQ ID NO: 9746	0.11 %	6
15	47	SEQ ID NO: 9747	0.07 %	4
16	66	SEQ ID NO: 9748	0.07 %	4
17	95	SEQ ID NO: 9749	0.07 %	4
18	104	SEQ ID NO: 9750	0.07 %	4
19	152	SEQ ID NO: 9751	0.07 %	4
20	158	SEQ ID NO: 9752	0.07 %	4

TABLE 26: Cloned sequences for *E.coli* expression

ORF	DNA length bp	Cloning	
		pET	pGEX
P28	537	NdeI / XhoI	
P65	1917	NheI / HindIII	
Nsp1A	2495	NheI / XhoI	
Nsp1B	2153	NdeI / XhoI	
Nsp1C	2612	NdeI / XhoI	
Nsp2A	431	NdeI / XhoI	BamHI / XhoI
Nsp2B	426	NdeI / XhoI	BamHI / XhoI
Nsp3	870	NdeI / XhoI	
Nsp4	249	NdeI / XhoI	BamHI / XhoI
Nsp5	594	NheI / XhoI	
Nsp6	339	NdeI / XhoI	BamHI / XhoI
Nsp7	417	NdeI / XhoI	BamHI / XhoI
Nsp9A	1385	NheI /XhoI	
Nsp9B	1409	NdeI / XhoI	
Nsp10	1803	NheI / XhoI	
Nsp11	1581	NdeI / XhoI	
Nsp12	1038	NdeI / HindIII	
Nsp13	897	NdeI / XhoI	
Spike (S1)	1946	NdeI / XhoI	
Spike (S2)	1598	NdeI / XhoI	
Spike (S1-S2)	3545	NdeI / XhoI	
HR1	287	NdeI / XhoI	BamHI / XhoI
HR2	146	NdeI / XhoI	BamHI / XhoI
ORF3Δ100	525	NdeI / XhoI	
ORF4	465	NdeI / XhoI	
Envelope (E)	231	NdeI / XhoI	BamHI / XhoI
Matrix (M)Δ100	366	NdeI / XhoI	BamHI / XhoI
ORF7Δ18	137	NdeI / XhoI	BamHI / XhoI
ORF8	369	NdeI / XhoI	BamHI / XhoI
ORF9	135	NdeI / XhoI	BamHI / XhoI
ORF10	120	NheI / XhoI	BamHI / XhoI
ORF11	255	NdeI / XhoI	BamHI / XhoI
Nucleocapsid (N)	1269	NdeI / EcoRI	
ORF12	297	NdeI / EcoRI	BamHI / EcoRI

TABLE 27: Primers

ORF	Forward primer	Reverse primer
P28	9803	9818
P65	9804	9819
Nsp1A	9805	9820
Nsp1B	9806	9821
Nsp1C	9807	9822
Nsp2 + Nsp3	9808	9823
Nsp4 to Nsp7	9809	9824
Nsp9A	9810	9825
Nsp9B	9811	9826
Nsp10	9812	9827
Nsp11	9813	9828
Nsp12-Nsp13	9814	9829
ORF3-ORF4	9815	9830
Env-ORF10	9816	9831
ORF11-ORF12	9817	9832

TABLE 28: Primers

ORF	Forward primer	Reverse primer
Nsp2A	SEQ ID NO: 9833	SEQ ID NO: 9858
Nsp2B	SEQ ID NO: 9834	SEQ ID NO: 9859
Nsp3	SEQ ID NO: 9835	SEQ ID NO: 9860
Nsp4	SEQ ID NO: 9836	SEQ ID NO: 9861
Nsp5	SEQ ID NO: 9837	SEQ ID NO: 9862
Nsp6	SEQ ID NO: 9838	SEQ ID NO: 9863
Nsp7	SEQ ID NO: 9839	SEQ ID NO: 9864
Nsp12	SEQ ID NO: 9840	SEQ ID NO: 9865
Nsp13	SEQ ID NO: 9841	SEQ ID NO: 9866
Spike S1	SEQ ID NO: 9842	SEQ ID NO: 9867
Spike S2	SEQ ID NO: 9843	SEQ ID NO: 9868
Spike S1-S2	SEQ ID NO: 9844	SEQ ID NO: 9869
HR1	SEQ ID NO: 9845	SEQ ID NO: 9870
HR2	SEQ ID NO: 9846	SEQ ID NO: 9871
Orf3Δ100	SEQ ID NO: 9847	SEQ ID NO: 9872
Orf4	SEQ ID NO: 9848	SEQ ID NO: 9873
Env E	SEQ ID NO: 9849	SEQ ID NO: 9874
Matrix MΔ100	SEQ ID NO: 9850	SEQ ID NO: 9875
Orf7Δ18	SEQ ID NO: 9851	SEQ ID NO: 9876
Orf8	SEQ ID NO: 9852	SEQ ID NO: 9877
Orf9	SEQ ID NO: 9853	SEQ ID NO: 9878
Orf10	SEQ ID NO: 9854	SEQ ID NO: 9879
Orf11	SEQ ID NO: 9855	SEQ ID NO: 9880
Nucleocapsid N	SEQ ID NO: 9856	SEQ ID NO: 9881
Orf12	SEQ ID NO: 9857	SEQ ID NO: 9882

TABLE 29: Cloning, purification and expression in *E.coli*

SARS CoV ORFs	M.W Kd	cloning	Expr.	purification as
P28	19,7	+	+	his sol
P65	70,3	+	+	his sol
Nsp1A (N-term)	91,6	+	+	his ins
Nsp1B (core)	80,8	+	-	
Nsp1C (C-term)	95,3	+	-	
Nsp2A (N-term)	15,8	+	+	his ins
Nsp2B (C-term)	15,5	+	+	his sol
Nsp3	31,9	+	-	
Nsp4	9,1	+	+	his sol
Nsp5	21,8	+	+	his sol
Nsp6	12,4	+	+	his sol
Nsp7	15,3	+	+	his ins
Nsp9A (N-term)	50,8	+	-	
Nsp9B (C-term)	51,6	+	+	his ins
Nsp10	66	-		
Nsp11	58	-		
Nsp12	38	-		
Nsp13	32,7	+	+	his ins
Spike (S1-his)	71,3	+	+	his ins
Spike (S2-his)	58,6	+	-	
Spike (S1S2-his)	130	+	+	his ins
HR1	11	+	+	his ins
HR2	5,4	+	+	his sol
ORF3 Δ100[1]	19,1	+	-	
ORF4	16,9	+	+	his ins (trimer)
Envelope (E)	34,3	+	+	gst ins (IB)
Matrix (M) Δ100	13,3	+	+	his ins
ORF7Δ18[2]	31	+	+	gst sol
ORF8	39,5	+	+	gst ins (IB)
ORF9	30,8	+	+	gst sol
ORF10	30,3	+	+	gst ins (IB)
ORF11	35,2	+	+	gst ins (IB)
Nucleocapsid (N)	43,6	+	+	his ins
ORF12	36,7	+	+	his ins

TABLE 30: *E.coli* expression, purity and yield

Protein	Tag	Purity (%)	Yield (mg/l)
Nsp2A (N-term)	His	95	1.7
Nsp2B (C-term)	His	95	4.1
Nsp4	His	95	12.6
Nsp5	His	95	5.88
Nsp6	His	95	8.1
P28	His	95	1
P65	His	80	0.553
HR2	His	95	11.9
HR1	His	80	2.64
Nsp1A	His	95	0.267
Spike S1-S2	His	80	0.381
Matrix M	His	85	12.4
ORF7	GST	85	4.9

TABLE 31: Primers

SEQ ID NO:	Rank	Model	Local	(Position)
10235	F1	1	1	(106)
10236	F2	2	1	(728)
10237	F3	3	1	(112)
10238	F4	5	2	(1331)
10239	F5	6	1	(12)
10240	F6	6	1	(346)
10241	F7	8	1	(904)
10242	F8	9	1	(1016)
10243	F9	9	1	(1015)
10244	F10	9	1	(719)
10245	F11	9	1	(720)
10246	F12	10	1	(724)
10247	R1	2	1	(1283)
10248	R2	4	1	(756)
10249	R3	4	1	(758)
10250	R4	5	2	(259)
10251	R5	6	1	(54)
10252	R6	7	1	(648)
10253	R7	8	1	(948)
10254	R8	8	1	(260)
10255	R9	9	1	(1282)
10256	R10	9	1	(950)
10257	R11	9	1	(756)
10258	R12	10	1	(132)

TABLE 32: Primers

Primers List: (forward)

Rank	Scores Model	Local	Sequence	(Position)
F1	7	1	SEQ ID NO: 10352	(290)
F2	7	1	SEQ ID NO: 10353	(291)
F3	7	1	SEQ ID NO: 10354	(294)
F4	7	1	SEQ ID NO: 10355	(292)
F5	7	1	SEQ ID NO: 10356	(293)
F6	9	1	SEQ ID NO: 10357	(198)
F7	9	1	SEQ ID NO: 10358	(199)
F8	10	1	SEQ ID NO: 10359	(33)
F9	11	1	SEQ ID NO: 10360	(200)
F10	11	1	SEQ ID NO: 10361	(299)
F11	12	1	SEQ ID NO: 10362	(298)
F12	12	1	SEQ ID NO: 10363	(297)
F13	14	1	SEQ ID NO: 10364	(35)
F14	14	1	SEQ ID NO: 10365	(34)
F15	16	1	SEQ ID NO: 10366	(300)
F16	17	1	SEQ ID NO: 10367	(295)
F17	17	1	SEQ ID NO: 10368	(296)
F18	17	1	SEQ ID NO: 10369	(175)
F19	17	1	SEQ ID NO: 10370	(36)
F20	20	1	SEQ ID NO: 10371	(202)
F21	20	1	SEQ ID NO: 10372	(201)
F22	28	1	SEQ ID NO: 10373	(204)
F23	28	1	SEQ ID NO: 10374	(203)
F24	29	1	SEQ ID NO: 10375	(269)
F25	29	1	SEQ ID NO: 10376	(268)

Primers List (reverse)

Rank	Model	Local	Sequence	(Position)
R1	7	1	SEQ ID NO: 10377	(337)
R2	9	1	SEQ ID NO: 10378	(229)
R3	11	1	SEQ ID NO: 10379	(230)
R4	11	1	SEQ ID NO: 10380	(338)
R5	12	1	SEQ ID NO: 10381	(207)
R6	12	1	SEQ ID NO: 10382	(338)
R7	13	1	SEQ ID NO: 10383	(231)
R8	14	1	SEQ ID NO: 10384	(80)
R9	14	1	SEQ ID NO: 10385	(232)
R10	15	1	SEQ ID NO: 10386	(82)
R11	16	1	SEQ ID NO: 10387	(340)
R12	17	1	SEQ ID NO: 10388	(83)
R13	17	1	SEQ ID NO: 10389	(206)
R14	17	1	SEQ ID NO: 10390	(82)
R15	17	1	SEQ ID NO: 10391	(337)
R16	18	1	SEQ ID NO: 10392	(341)
R17	20	1	SEQ ID NO: 10393	(340)
R18	20	1	SEQ ID NO: 10394	(233)
R19	21	1	SEQ ID NO: 10395	(79)
R20	22	1	SEQ ID NO: 10396	(213)
R21	28	1	SEQ ID NO: 10397	(236)
R22	29	1	SEQ ID NO: 10398	(317)
R23	32	1	SEQ ID NO: 10399	(391)
R24	35	1	SEQ ID NO: 10400	(57)
R25	36	1	SEQ ID NO: 10401	(237)

Primers List (left part): SEQ ID NO^{S}: 10402-10433	Primers List (right part): SEQ ID NO^{S}: 10434-10464
Primers List (forward): SEQ ID NO^{S}: 10465-10484	Primers List (reverse): SEQ ID NO^{S}: 10485-10504

TABLE 33: Primers

Primers List (forward)

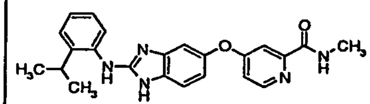
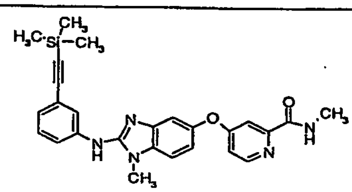
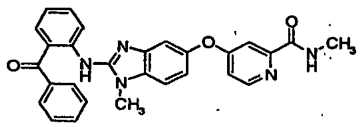
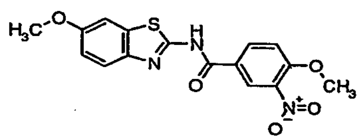
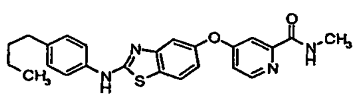
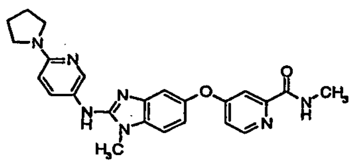
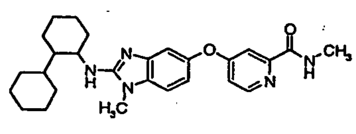
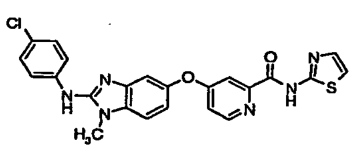
Rank	Scores Model	Scores Local	Sequence	(Position)
F1	1	1	SEQ ID NO: 10580	(637)
F2	2	1	SEQ ID NO: 10581	(439)
F3	2	1	SEQ ID NO: 10582	(440)
F4	3	1	SEQ ID NO: 10583	(729)
F5	4	1	SEQ ID NO: 10584	(696)
F6	4	1	SEQ ID NO: 10585	(697)
F7	4	1	SEQ ID NO: 10586	(111)
F8	5	1	SEQ ID NO: 10587	(867)
F9	5	1	SEQ ID NO: 10588	(868)
F10	5	1	SEQ ID NO: 10589	(869)
F11	5	1	SEQ ID NO: 10590	(640)
F12	6	1	SEQ ID NO: 10591	(438)
F13	6	1	SEQ ID NO: 10592	(437)
F14	6	1	SEQ ID NO: 10593	(436)
F15	6	1	SEQ ID NO: 10594	(732)
F16	6	1	SEQ ID NO: 10595	(635)
F17	6	1	SEQ ID NO: 10596	(457)
F18	6	1	SEQ ID NO: 10597	(458)
F19	6	1	SEQ ID NO: 10598	(636)
F20	7	1	SEQ ID NO: 10599	(854)
F21	7	1	SEQ ID NO: 10600	(855)
F22	7	1	SEQ ID NO: 10601	(581)
F23	7	1	SEQ ID NO: 10602	(853)
F24	7	1	SEQ ID NO: 10603	(342)
F25	7	1	SEQ ID NO: 10604	(343)
F26	7	1	SEQ ID NO: 10605	(112)
F27	7	1	SEQ ID NO: 10606	(94)
F28	7	1	SEQ ID NO: 10607	(642)
F29	8	1	SEQ ID NO: 10608	(638)
F30	8	1	SEQ ID NO: 10609	(639)
F31	8	1	SEQ ID NO: 10610	(730)
F32	8	1	SEQ ID NO: 10611	(641)
F33	8	1	SEQ ID NO: 10612	(731)
F34	8	1	SEQ ID NO: 10613	(326)
F35	8	1	SEQ ID NO: 10614	(325)
F36	9	1	SEQ ID NO: 10615	(517)
F37	9	1	SEQ ID NO: 10616	(701)
F38	9	1	SEQ ID NO: 10617	(208)
F39	9	1	SEQ ID NO: 10618	(209)
F40	9	1	SEQ ID NO: 10619	(702)
F41	9	1	SEQ ID NO: 10620	(210)
F42	10	1	SEQ ID NO: 10621	(634)
F43	10	1	SEQ ID NO: 10622	(694)
F44	10	1	SEQ ID NO: 10623	(693)
F45	10	1	SEQ ID NO: 10624	(728)
F46	10	1	SEQ ID NO: 10625	(695)
F47	10	1	SEQ ID NO: 10626	(95)
F48	11	1	SEQ ID NO: 10627	(455)
F49	11	1	SEQ ID NO: 10628	(456)
F50	11	1	SEQ ID NO: 10629	(454)

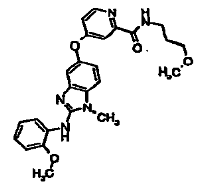
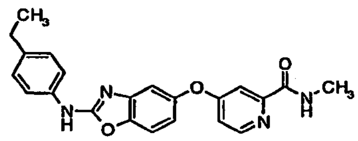
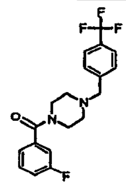
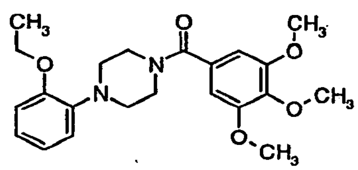
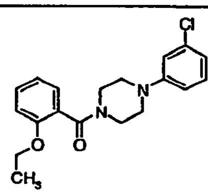
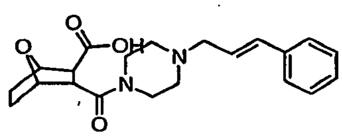
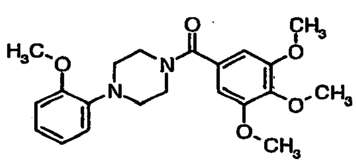
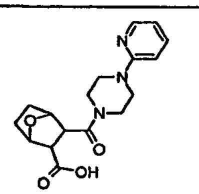
Primers List (reverse)

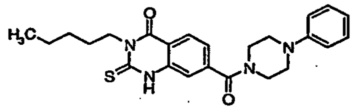
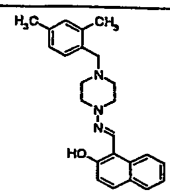
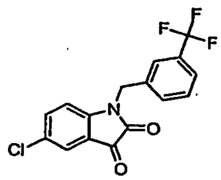
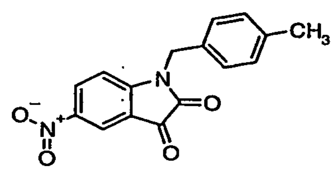
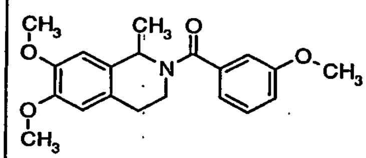
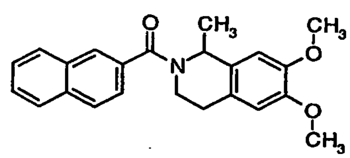
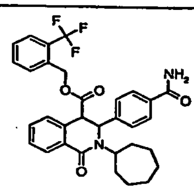
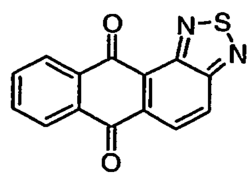
	Scores			
Rank	Model	Local	Sequence	(Position)
R1	1	1	SEQ ID NO: 10630	(367)
R2	1	1	SEQ ID NO: 10631	(666)
R3	2	1	SEQ ID NO: 10632	(464)
R4	3	1	SEQ ID NO: 10633	(669)
R5	3	1	SEQ ID NO: 10634	(750)
R6	4	1	SEQ ID NO: 10635	(720)
R7	4	1	SEQ ID NO: 10636	(465)
R8	4	1	SEQ ID NO: 10637	(370)
R9	4	1	SEQ ID NO: 10638	(668)
R10	4	1	SEQ ID NO: 10639	(135)
R11	5	1	SEQ ID NO: 10640	(901)
R12	5	1	SEQ ID NO: 10641	(667)
R13	6	1	SEQ ID NO: 10642	(609)
R14	6	1	SEQ ID NO: 10643	(464)
R15	6	1	SEQ ID NO: 10644	(665)
R16	6	1	SEQ ID NO: 10645	(486)
R17	6	1	SEQ ID NO: 10646	(356)
R18	6	1	SEQ ID NO: 10647	(758)
R19	7	1	SEQ ID NO: 10648	(366)
R20	7	1	SEQ ID NO: 10649	(368)
R21	7	1	SEQ ID NO: 10650	(136)
R22	7	1	SEQ ID NO: 10651	(675)
R23	7	1	SEQ ID NO: 10652	(366)
R24	7	1	SEQ ID NO: 10653	(608)
R25	7	1	SEQ ID NO: 10654	(884)
R26	7	1	SEQ ID NO: 10655	(120)
R27	8	1	SEQ ID NO: 10656	(355)
R28	8	1	SEQ ID NO: 10657	(671)
R29	8	1	SEQ ID NO: 10658	(756)
R30	8	1	SEQ ID NO: 10659	(751)
R31	8	1	SEQ ID NO: 10660	(666)
R32	9	1	SEQ ID NO: 10661	(242)
R33	9	1	SEQ ID NO: 10662	(543)
R34	9	1	SEQ ID NO: 10663	(724)
R35	9	1	SEQ ID NO: 10664	(482)
R36	10	1	SEQ ID NO: 10665	(121)
R37	10	1	SEQ ID NO: 10666	(662)
R38	10	1	SEQ ID NO: 10667	(750)
R39	10	1	SEQ ID NO: 10668	(719)
R40	10	1	SEQ ID NO: 10669	(242)
R41	11	1	SEQ ID NO: 10670	(484)
R42	11	1	SEQ ID NO: 10671	(375)
R43	11	1	SEQ ID NO: 10672	(728)
R44	11	1	SEQ ID NO: 10673	(373)
R45	11	1	SEQ ID NO: 10674	(998)
R46	11	1	SEQ ID NO: 10675	(486)
R47	12	1	SEQ ID NO: 10676	(881)
R48	12	1	SEQ ID NO: 10677	(882)
R49	12	1	SEQ ID NO: 10678	(244)
R50	12	1	SEQ ID NO: 10679	(1003)

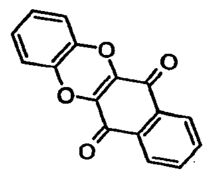
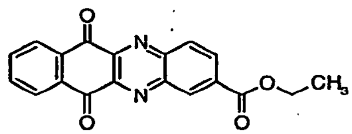
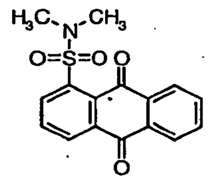
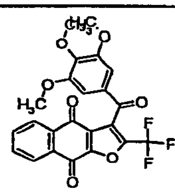
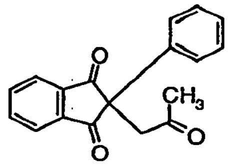
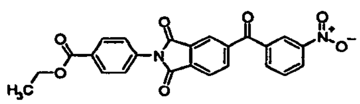
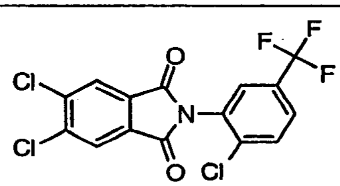
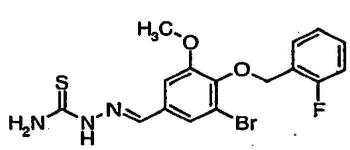
Primers List (left part): SEQ ID NO^{S}: 10680-10974	Primers List (right part): SEQ ID NO^{S}: 10975-11282
Primers List (forward): SEQ ID NO^{S}: 11283-11302	Primers List (reverse): SEQ ID NO^{S}: 11303-11322

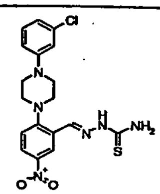
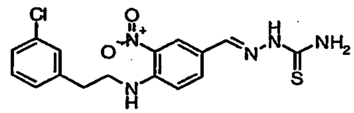
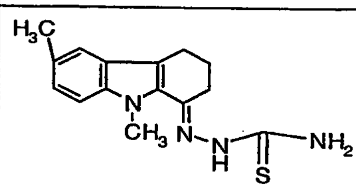
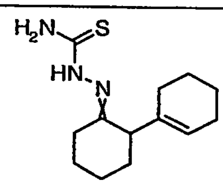
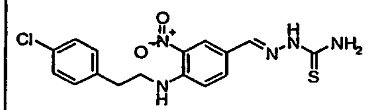
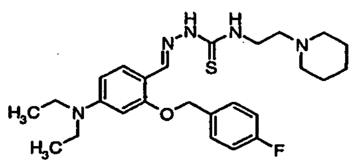
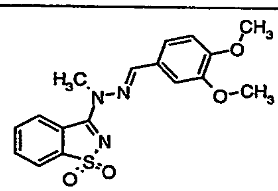
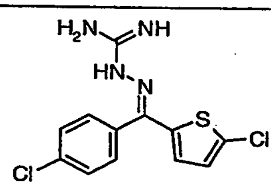
TABLE 34

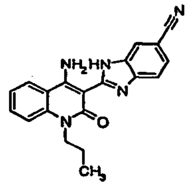
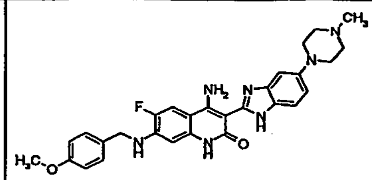
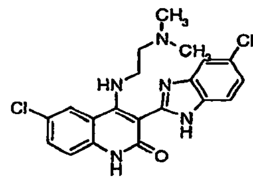
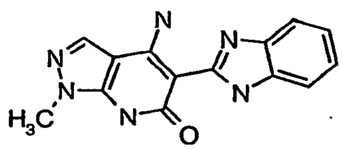
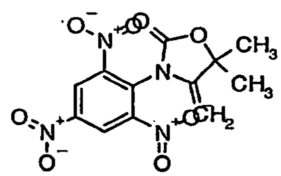
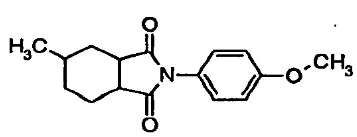
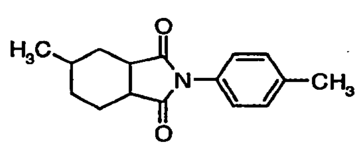
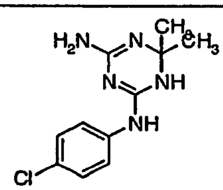
Compound #	Structure	Name	MH+
1		N-methyl-4-[(2-{[2-(1-methylethyl)phenyl]amino}-1H-benzimidazol-5-yl)oxy]pyridine-2-carboxamide	402.5
2		N-methyl-4-{[1-methyl-2-({3-[(trimethylsilyl)ethynyl]phenyl}amino)-1H-benzimidazol-5-yl]oxy}pyridine-2-carboxamide	470.6
3		N-methyl-4-[(1-methyl-2-{[2-(phenylcarbonyl)phenyl]amino}-1H-benzimidazol-5-yl)oxy]pyridine-2-carboxamide	478.5
4		4-(methyloxy)-N-[6-(methyloxy)-1,3-benzothiazol-2-yl]-3-nitrobenzamide	360.4
5		4-({2-[(4-butylphenyl)amino]-1,3-benzothiazol-5-yl}oxy)-N-methylpyridine-2-carboxamide	433.5
6		N-methyl-4-({1-methyl-2-[(6-pyrrolidin-1-ylpyridin-3-yl)amino]-1H-benzimidazol-5-yl}oxy)pyridine-2-carboxamide	444.5
7		4-({2-[1,1'-bi(cyclohexyl)-2-ylamino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-methylpyridine-2-carboxamide	462.6
8		4-({2-[(4-chlorophenyl)amino]-1-methyl-1H-benzimidazol-5-yl}oxy)-N-1,3-thiazol-2-ylpyridine-2-carboxamide	477.9

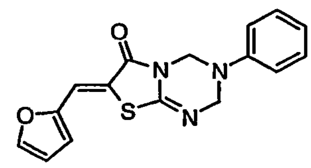
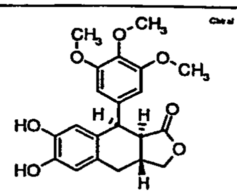
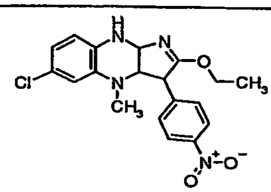
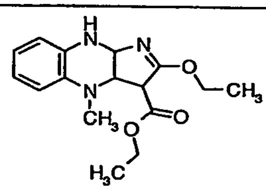
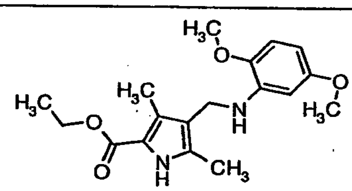
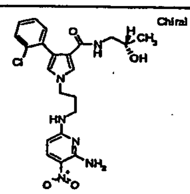
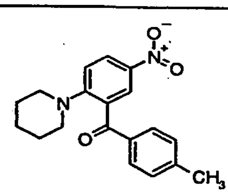
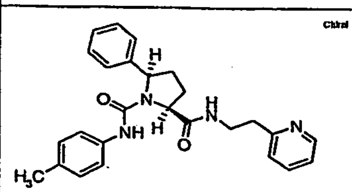
9		4-[(1-methyl-2-{[2-(methyloxy)phenyl]amino}-1H-benzimidazol-5-yl)oxy]-N-[3-(methyloxy)propyl]pyridine-2-carboxamide	462.5
10		4-({2-[(4-ethylphenyl)amino]-1,3-benzoxazol-5-yl}oxy)-N-methylpyridine-2-carboxamide	389.4
11		1-[(3-fluorophenyl)carbonyl]-4-{[4-(trifluoromethyl)phenyl]methyl}piperazine	367.4
12		1-[2-(ethyloxy)phenyl]-4-{[3,4,5-tris(methyloxy)phenyl]carbonyl}piperazine	401.5
13		1-(3-chlorophenyl)-4-{[2-(ethyloxy)phenyl]carbonyl}piperazine	345.8
14		3-({4-[(2E)-3-phenylprop-2-enyl]piperazin-1-yl}carbonyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid	371.4
15		1-[2-(methyloxy)phenyl]-4-{[3,4,5-tris(methyloxy)phenyl]carbonyl}piperazine	387.4
16		3-[(4-pyridin-2-ylpiperazin-1-yl)carbonyl]-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid	332.4

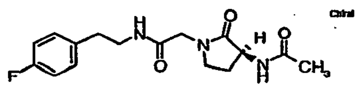
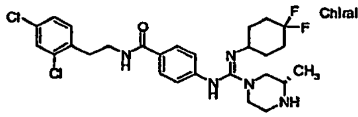
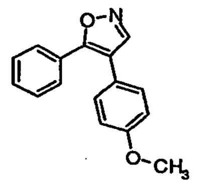
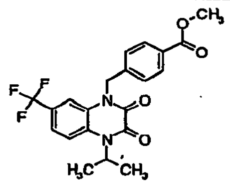
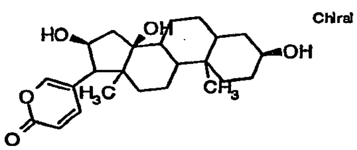
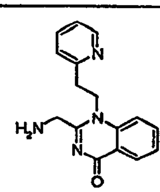
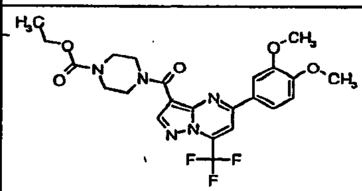
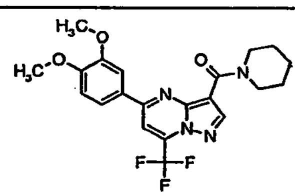
17		3-pentyl-7-[(4-phenylpiperazin-1-yl)carbonyl]-2-thioxo-2,3-dihydroquinazolin-4(1H)-one	437.6
18		1-[(E)-({4-[(2,4-dimethylphenyl)methyl]piperazin-1-yl}imino)methyl]naphthalen-2-ol	374.5
19		5-chloro-1-{[3-(trifluoromethyl)phenyl]methyl}-1H-indole-2,3-dione	340.7
20		1-[(4-methylphenyl)methyl]-5-nitro-1H-indole-2,3-dione	297.3
21		1-methyl-6,7-bis(methyloxy)-2-{[3-(methyloxy)phenyl]carbonyl}-1,2,3,4-tetrahydroisoquinoline	342.4
22		1-methyl-6,7-bis(methyloxy)-2-(naphthalen-2-ylcarbonyl)-1,2,3,4-tetrahydroisoquinoline	362.4
23		[2-(trifluoromethyl)phenyl]methyl 3-[4-(aminocarbonyl)phenyl]-2-cycloheptyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate	565.6
24		anthra[1,2-c][1,2,5]thiadiazole-6,11-dione	267.3

25		benzo[b]oxanthrene-6,11-dione	265.2
26		ethyl 6,11-dioxo-6,11-dihydrobenzo[b]phenazine-2-carboxylate	333.3
27		N,N-dimethyl-9,10-dioxo-9,10-dihydroanthracene-1-sulfonamide	316.3
28		2-(trifluoromethyl)-3-{[3,4,5-tris(methyloxy)phenyl]carbonyl}naphtho[2,3-b]furan-4,9-dione	461.4
29		2-(2-oxopropyl)-2-phenyl-1H-indene-1,3(2H)-dione	279.3
30		ethyl 4-{5-[(3-nitrophenyl)carbonyl]-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl}benzoate	445.4
31		5,6-dichloro-2-[2-chloro-5-(trifluoromethyl)phenyl]-1H-isoindole-1,3(2H)-dione	395.6
32		3-bromo-4-{[(2-fluorophenyl)methyl]oxy}-5-(methyloxy)benzaldehyde thiosemicarbazone	413.3

33		2-[4-(3-chlorophenyl)piperazin-1-yl]-5-nitrobenzaldehyde thiosemicarbazone	419.9
34		4-{[2-(3-chlorophenyl)ethyl]amino}-3-nitrobenzaldehyde thiosemicarbazone	378.9
35		(1E)-6,9-dimethyl-2,3,4,9-tetrahydro-1H-carbazol-1-one thiosemicarbazone	287.4
36		(2E)-1,1'-bi(cyclohexan)-1-en-2-one thiosemicarbazone	252.4
37		4-{[2-(4-chlorophenyl)ethyl]amino}-3-nitrobenzaldehyde thiosemicarbazone	378.9
38		4-(diethylamino)-2-{[(4-fluorophenyl)methyl]oxy}benzaldehyde N-(2-piperidin-1-ylethyl)thiosemicarbazone	486.7
39		3,4-bis(methyloxy)benzaldehyde (1,1-dioxido-1,2-benzisothiazol-3-yl)(methyl)hydrazone	360.4
40		(2E)-2-[(4-chlorophenyl)(5-chlorothien-2-yl)methylidene]hydrazinecarboximidamide	314.2

41		2-(4-amino-2-oxo-1-propyl-1,2-dihydroquinolin-3-yl)-1H-benzimidazole-6-carbonitrile	344.4
42		4-amino-6-fluoro-7-({[4-(methyloxy)phenyl]methyl}amino)-3-[5-(4-methylpiperazin-1-yl)-1H-benzimidazol-2-yl]quinolin-2(1H)-one	528.6
43		6-chloro-3-(5-chloro-1H-benzimidazol-2-yl)-4-{[2-(dimethylamino)ethyl]amino}quinolin-2(1H)-one	417.3
44		4-amino-5-(1H-benzimidazol-2-yl)-1-methyl-1,7-dihydro-6H-pyrazolo[3,4-b]pyridin-6-one	281.3
45		5,5-dimethyl-4-methylidene-3-(2,4,6-trinitrophenyl)-1,3-oxazolidin-2-one	339.2
46		5-methyl-2-[4-(methyloxy)phenyl]hexahydro-1H-isoindole-1,3(2H)-dione	274.3
47		5-methyl-2-(4-methylphenyl)hexahydro-1H-isoindole-1,3(2H)-dione	258.3
48		N~2~-(4-chlorophenyl)-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine	252.7

49		(7Z)-7-(furan-2-ylmethylidene)-3-phenyl-3,4-dihydro-2H-[1,3]thiazolo[3,2-a][1,3,5]triazin-6(7H)-one	312.4
50		(3aR,9R,9aR)-6,7-dihydroxy-9-[3,4,5-tris(methyloxy)phenyl]-3a,4,9,9a-tetrahydronaphtho[2,3-c]furan-1(3H)-one	387.4
51		6-chloro-2-(ethyloxy)-4-methyl-3-(4-nitrophenyl)-3a,4,9,9a-tetrahydro-3H-pyrrolo[2,3-b]quinoxaline	387.8
52		ethyl 2-(ethyloxy)-4-methyl-3a,4,9,9a-tetrahydro-3H-pyrrolo[2,3-b]quinoxaline-3-carboxylate	304.4
53		ethyl 4-({[2,5-bis(methyloxy)phenyl]amino}methyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate	333.4
54		1-{3-[(6-amino-5-nitropyridin-2-yl)amino]propyl}-4-(2-chlorophenyl)-N-[(2S)-2-hydroxypropyl]-1H-pyrrole-3-carboxamide	473.9
55		(4-methylphenyl)(5-nitro-2-piperidin-1-ylphenyl)methanone	325.4
56		(2S,5R)-N~1~-(4-methylphenyl)-5-phenyl-N~2~-(2-pyridin-2-ylethyl)pyrrolidine-1,2-dicarboxamide	429.5

57		2-[(3S)-3-(acetylamino)-2-oxopyrrolidin-1-yl]-N-[2-(4-fluorophenyl)ethyl]acetamide	322.4
58		N-[2-(2,4-dichlorophenyl)ethyl]-4-({(Z)-[(4,4-difluorocyclohexyl)imino][(3S)-3-methylpiperazin-1-yl]methyl}amino)benzamide	553.5
59		4-[4-(methyloxy)phenyl]-5-phenylisoxazole	252.3
60		methyl 4-{[4-(1-methylethyl)-2,3-dioxo-7-(trifluoromethyl)-3,4-dihydroquinoxalin-1(2H)-yl]methyl}benzoate	421.4
61		(3beta,16beta)-3,14,16-trihydroxybufa-20,22-dienolide	403.5
62		2-(aminomethyl)-1-(2-pyridin-2-ylethyl)quinazolin-4(1H)-one	281.3
63		ethyl 4-{[5-[3,4-bis(methyloxy)phenyl]-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]carbonyl}piperazine-1-carboxylate	508.5
64		5-[3,4-bis(methyloxy)phenyl]-3-(piperidin-1-ylcarbonyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine	435.4

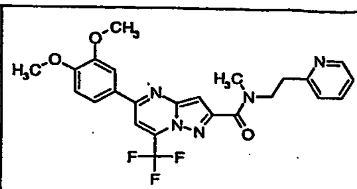
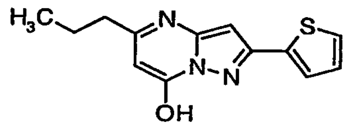
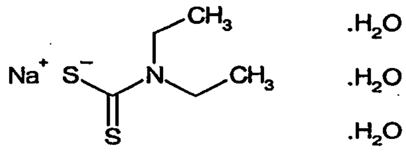
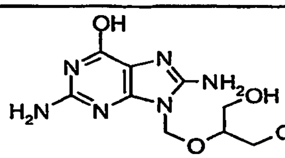
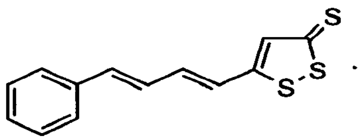
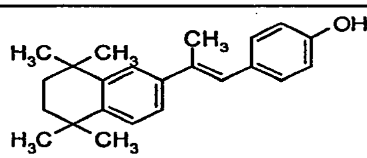
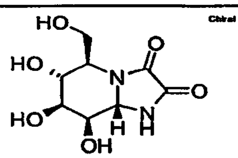
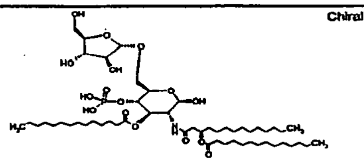
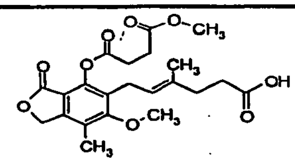
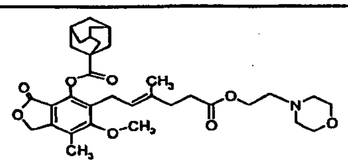
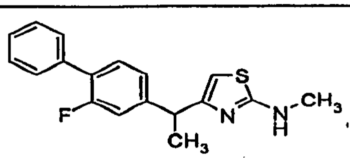
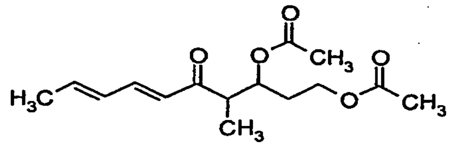
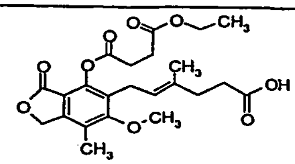
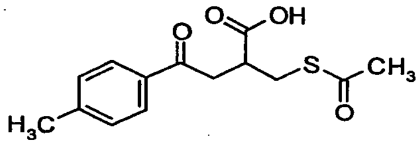
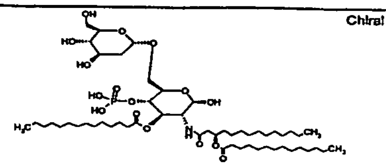
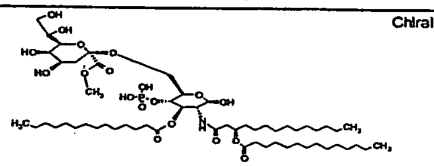
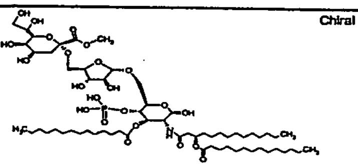
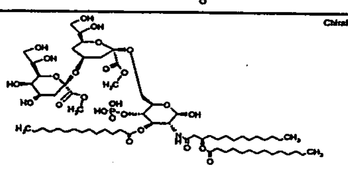
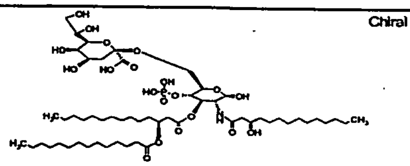
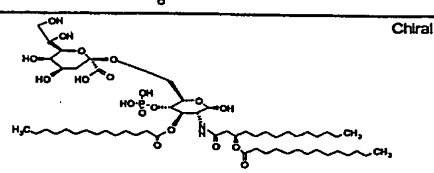
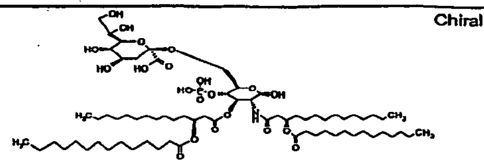
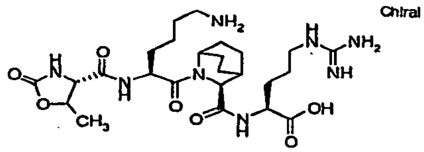
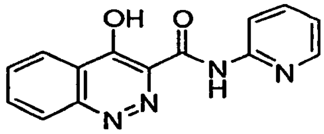
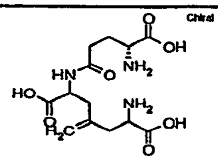
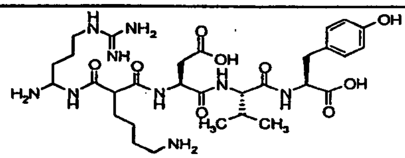
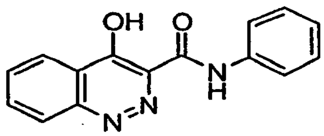
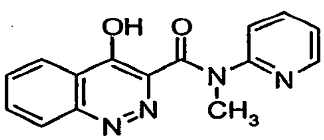
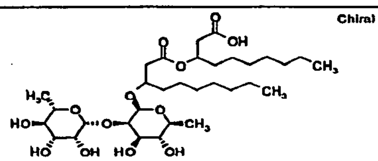
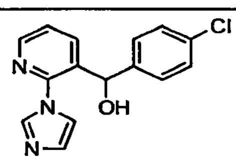
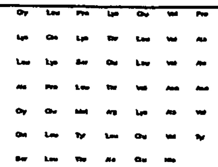
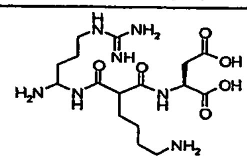
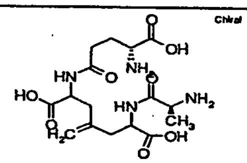
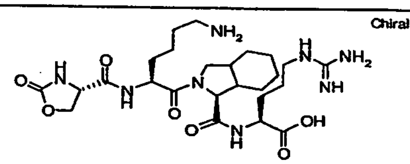
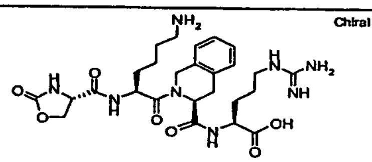
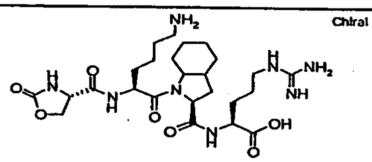
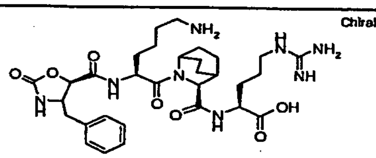
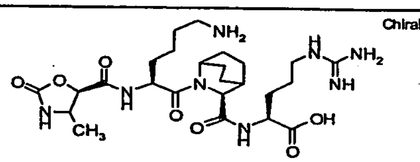
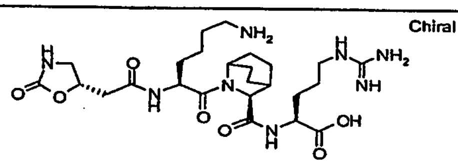
65		5-[3,4-bis(methyloxy)phenyl]-N-methyl-N-(2-pyridin-2-ylethyl)-7-(trifluoromethyl)pyrazolo[1,5-a]pyrimidine-2-carboxamide	486.5
66		5-propyl-2-thien-2-ylpyrazolo[1,5-a]pyrimidin-7-ol	260.3

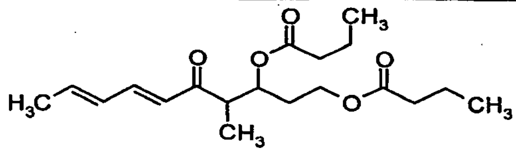
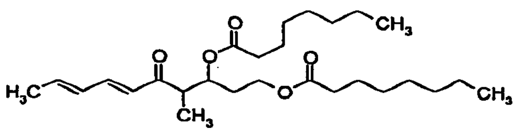
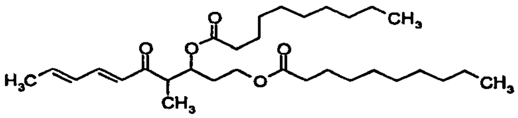
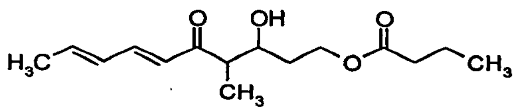
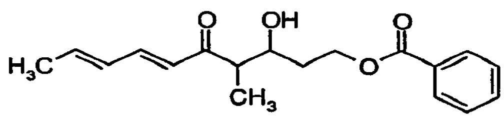
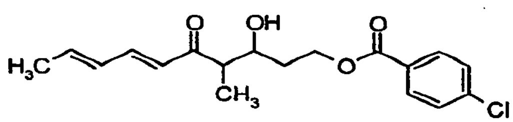
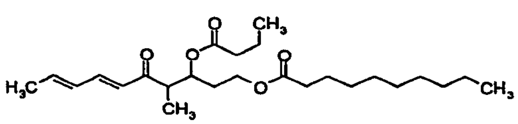
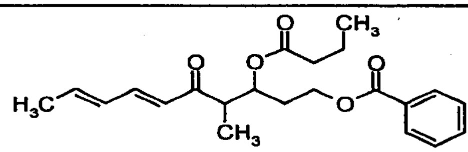
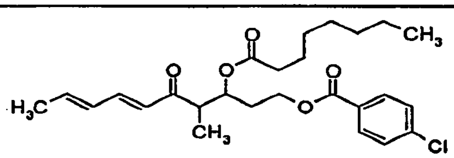
Table 35

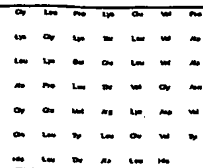
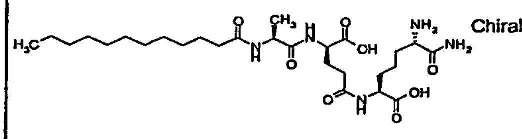
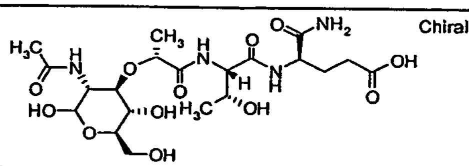
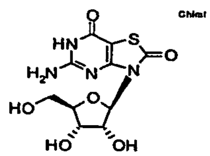
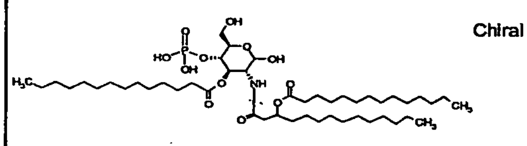
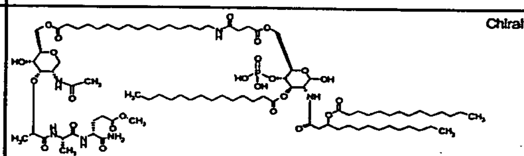
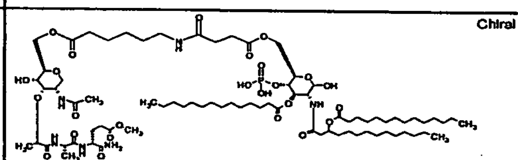
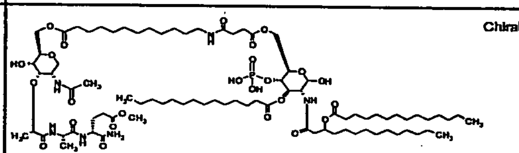
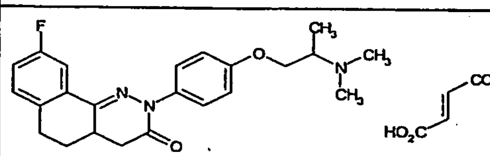
Compound #	Structure	Source	Literature Reference	Patent Number
1		Aventis Pasteur	1) Lang, J.-M.;Touraine, J.-L.;Trepo, C. et al. Lancet 1988, 2(8613): 702-5.	
2		Pfizer	Dong, M.K. et al. Pharmacologist 1988, 30(3): Abst 87.8.	ES 8602792
3		Mitsui Chemicals	Mizuno, O. et al. 4th Int Conf Immunopharmacol (May 15-19, Osaka) 1988, Abst WS6-3 .	EP 236929
4		Roche		EP 407788
5		Fujisawa	1) Iwami, M. et al. J Antibiot 1987, 40(5): 612-22.	JP 87161796
6		Novartis		FR 2604177
7		Roche Bioscience		US 4725622
8		Roche Bioscience		US 4727069
9		Sumitomo	1) Nishikaku, F. and Koga, Y. 4th Int Conf Immunopharmacol (May 15-19, Osaka) 1988, Abst WS6-8 .	EP 248399

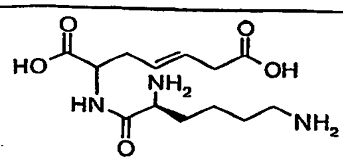
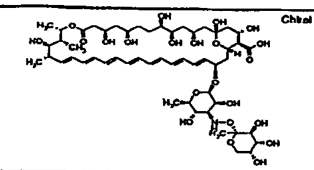
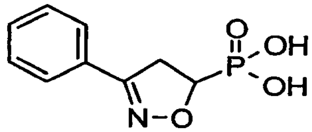
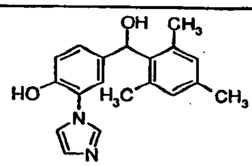
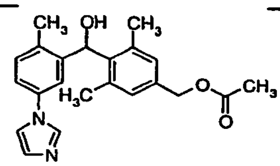
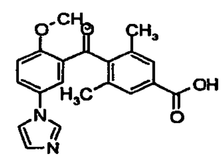
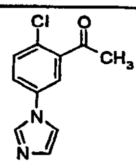
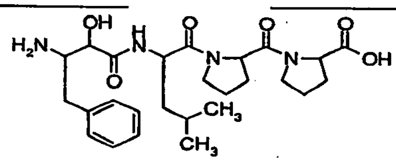
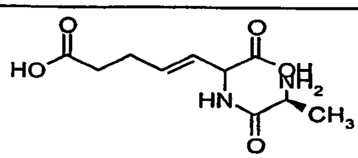
10		SSP		JP 88022053
11		Roche Bioscience		US 4725622
12		Taisho	1) Kameo, K. et al. Chem Pharm Bull 1988, 36(6): 2050-60.	EP 164101
13		Novartis		FR 2604177
14		Novartis		FR 2604177
15		Novartis		FR 2604177
16		Novartis		FR 2604177
17		Novartis		FR 2604177
18		Novartis		FR 2604177

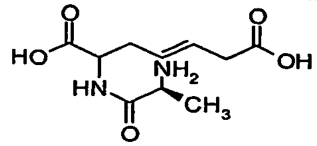
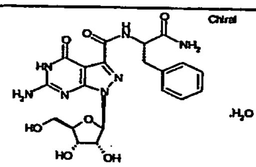
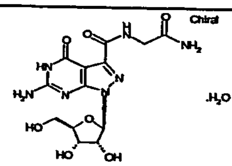
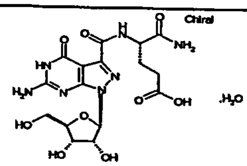
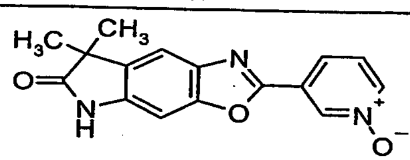
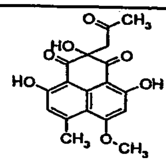
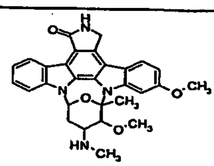
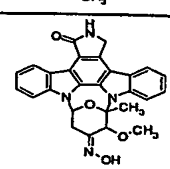
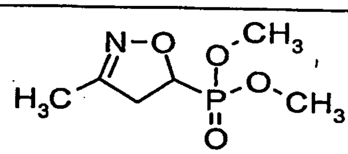
19		Novartis		FR 2604177
20		ADIR		AU 8811669
21		Pharmacia		AU 8810908
22		Aventis Pharma		EP 284461
23		Eniricerche		EP 282891
24		Pharmacia		AU 8810908
25		Pharmacia		AU 8810908
26		Abbott	Swanson, R.N. et al. 28th Intersci Conf Antimicrob Agents Chemother (Oct 23-26, Los Angeles) 1988, Abst 972.	
27		Mitsubishi Pharma		JP 88119425

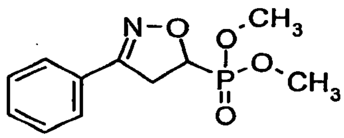
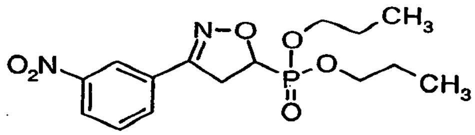
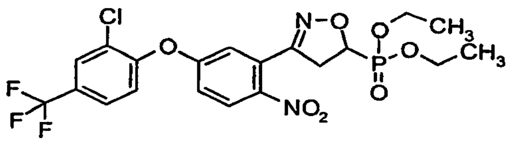
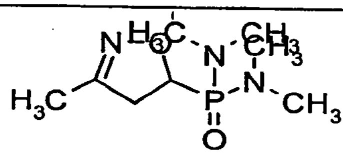
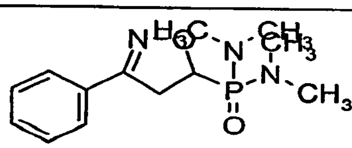
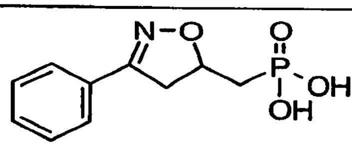
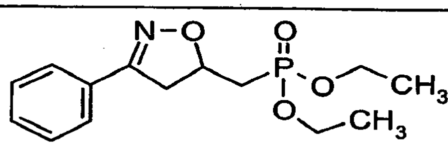
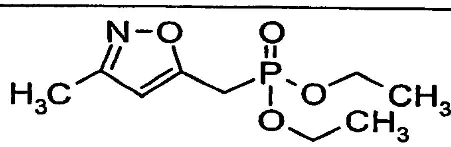
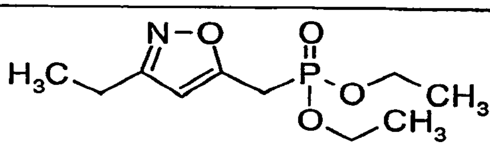
28		Ortho-McNeil		EP 292302
29		Eniricerche		EP 282891
30		Aventis Pharma		EP 284461
31		ADIR		AU 8811669
32		ADIR		AU 8811669
33		ADIR		AU 8811669
34		ADIR		AU 8811669
35		ADIR		AU 8811669
36		ADIR		AU 8811669

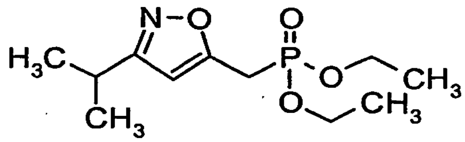
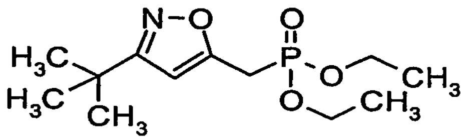
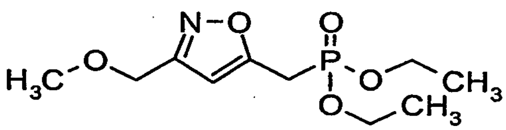
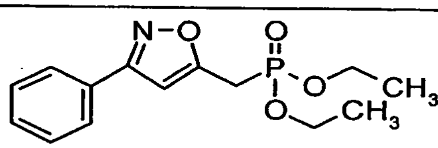
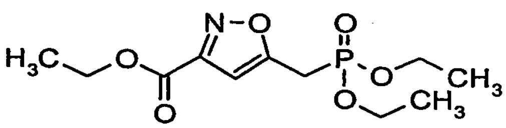
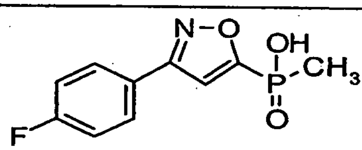
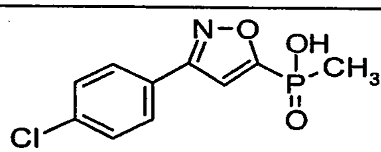
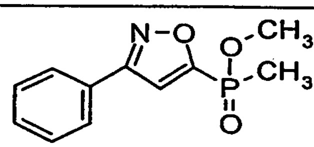
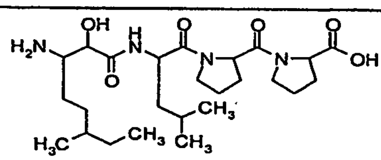
37		SSP		JP 88022053
38		SSP		JP 88022053
39		SSP		JP 88022053
40		SSP		JP 88022053
41		SSP		JP 88022053
42		SSP		JP 88022053
43		SSP		JP 88022053
44		SSP		JP 88022053
45		SSP		JP 88022053

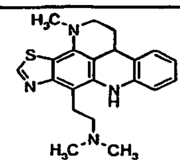
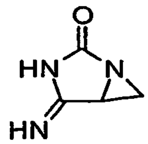
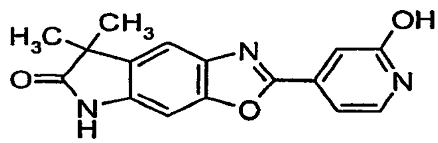
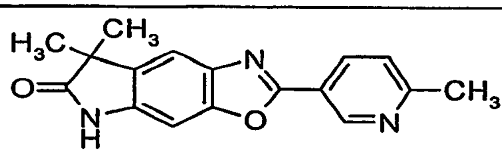
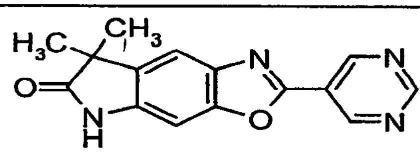
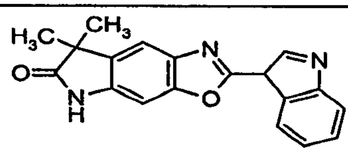
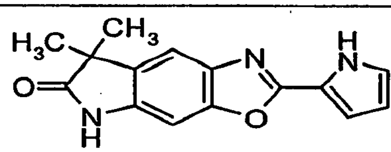
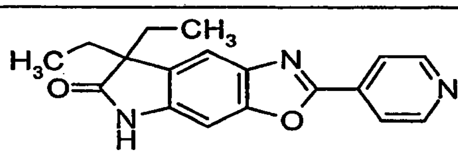
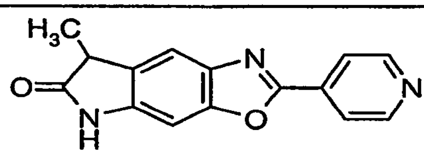
46		Ortho-McNeil		EP 300741
47		Aventis Pharma	1) Fizames, C. et al. 15th Int Cong Chemother (July 19-24, Istanbul) 1987, Abst 605 .	1) GB 2053231
48		Roche Bioscience	1) WHO Drug Inform 1988, 2(4): 227.	1) EP 135376
49		ICN	1) Sharma, B.S. et al. 3rd Intersci World Conf Inflamm, Antirheum Analg Immunomodul (March 15-18, Monte-Carlo) 1989, 9 .	EP 348446
50		Toho Yakuhin	1) Satoru, I. et al. Antivir Res 1988, 9(1-2): 37-46.	EP 188697
51		Gifu University	1) Hasegawa, A. et al. J Carbohyd Chem 1986, 5(3): 371-85.	
52		Gifu University		
53		Gifu University		
54		Mitsubishi Pharma		EP 351435

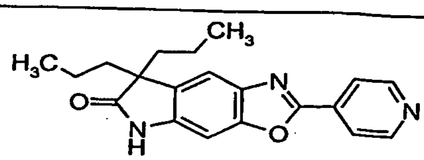
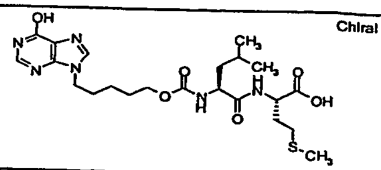
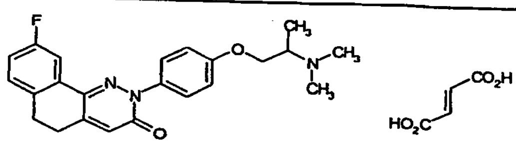
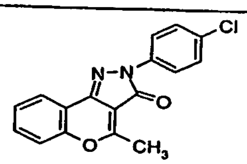
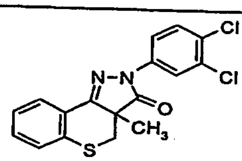
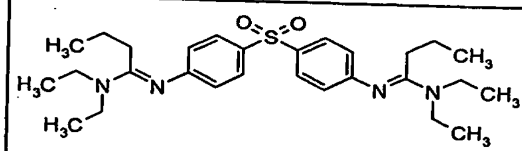
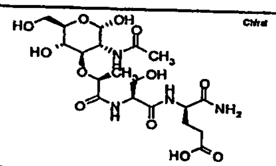
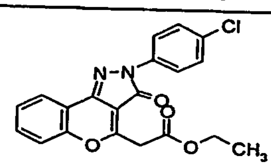
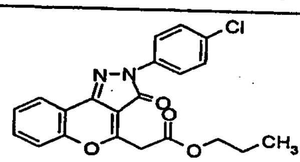
55		Aventis Pharma		AU 8824582
56		CNRS	1) Chiron, M. et al. Biochem Pharmacol 1988, 37(5): 827-36.	
57		Aventis Pharma	1) Schorlemmer, H.U.;Bartlett, R.R.;Dickneite, G.;Schwab, W.;Gebert, U.;Sedlacek, H.H.	AU 8824195
58		Mitsubishi Pharma		JP 88119425
59		Mitsubishi Pharma		JP 88119425
60		Mitsubishi Pharma		JP 88119425
61		Mitsubishi Pharma		JP 88119425
62		Microbial Chemistry Research Foundation		EP 310238
63		Aventis Pharma		AU 8824582

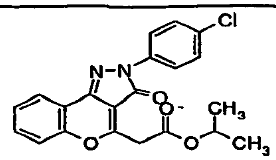
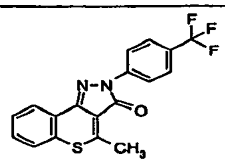
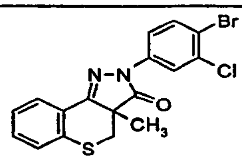
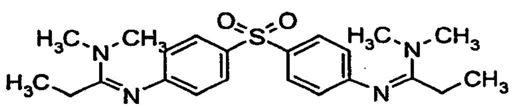
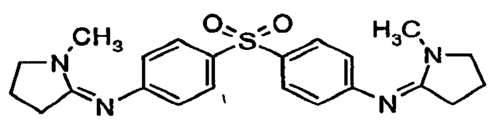
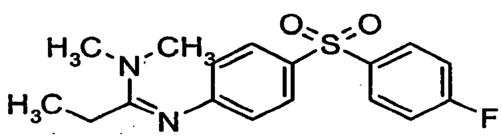
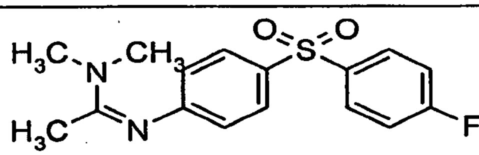
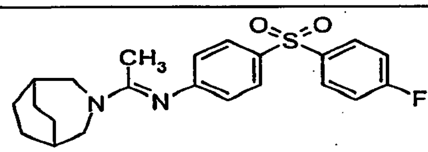
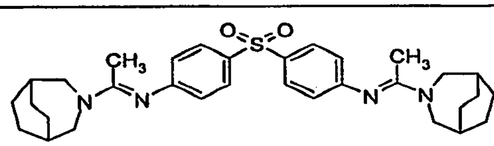
64		Aventis Pharma		AU 8824582
65		ICN	Ramasamy, K. et al. J Med Chem 1989, 32(8): 1905-9.	
66		ICN		
67		ICN		
68		Roche		AU 8931653
69		Fujisawa	Shibata, T. et al. J Antibiot 1989, 42(9): 1356-61.	
70		Takeda	Tanida, S. et al. J Antibiot 1989, 42(11): 1619.	
71		Takeda		
72		Aventis Pharma		AU 8824195

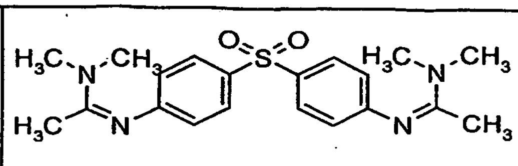
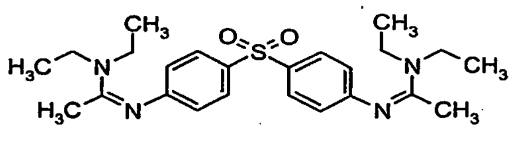
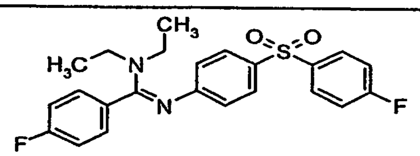
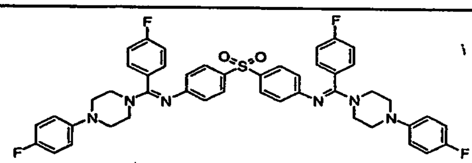
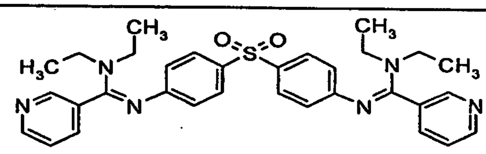
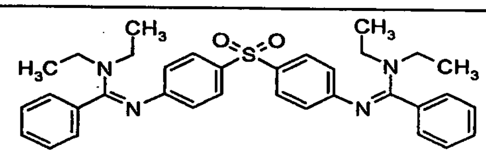
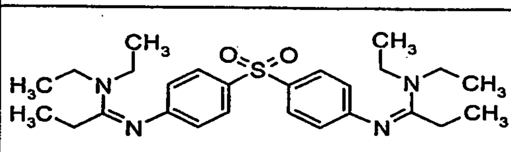
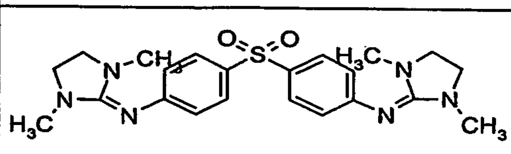
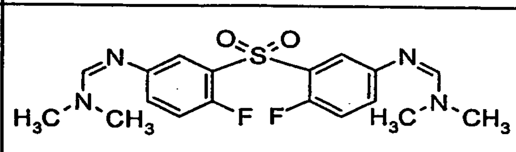
73		Aventis Pharma		AU 8824195
74		Aventis Pharma		AU 8824195
75		Aventis Pharma		AU 8824195
76		Aventis Pharma		AU 8824195
77		Aventis Pharma		AU 8824195
78		Aventis Pharma		AU 8824195
79		Aventis Pharma		AU 8824195
80		Aventis Pharma		AU 8824195
81		Aventis Pharma		AU 8824195

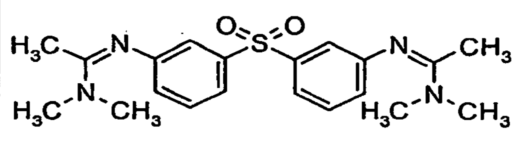
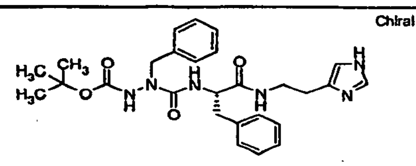
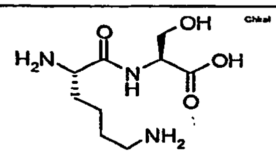
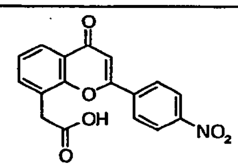
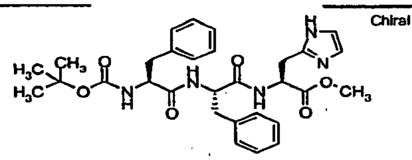
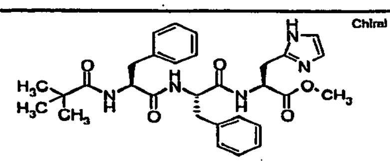
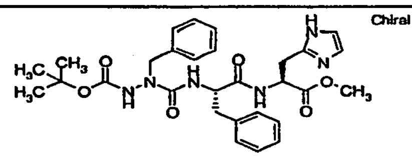
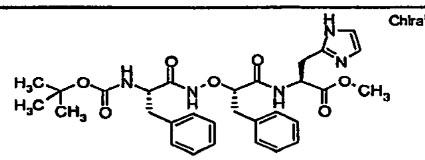
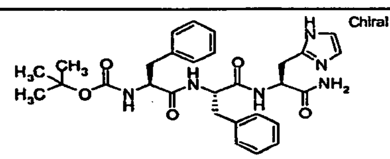
82		Aventis Pharma		AU 8824195
83		Aventis Pharma		AU 8824195
84		Aventis Pharma		AU 8824195
85		Aventis Pharma		AU 8824195
86		Aventis Pharma		AU 8824195
87		Aventis Pharma		AU 8824195
88		Aventis Pharma		AU 8824195
89		Aventis Pharma		AU 8824195
90		Microbial Chemistry Research Foundation		EP 310238

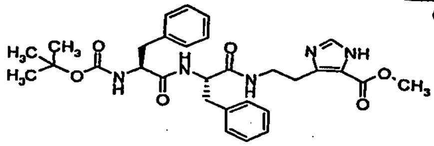
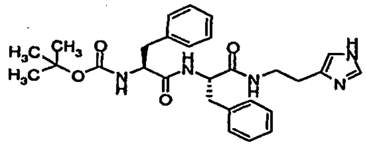
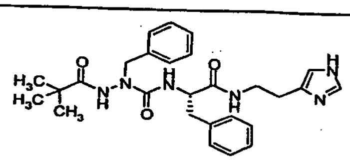
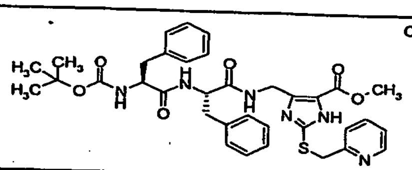
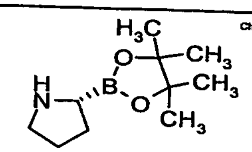
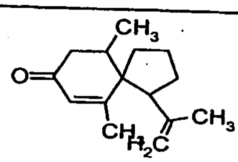
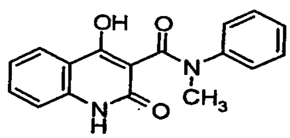
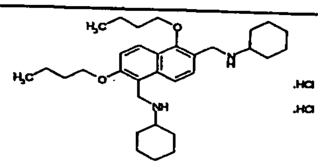
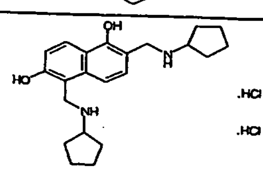
91		Harbor Branch Oceanographic Institution		EP 331320
92		Roche	1) Herrmann, D.B.J. 17th Int Cong Chemother (June 23-28, Berlin) 1991, Abst 838 .	EP 352652
93		Roche		AU 8931653
94		Roche		AU 8931653
95		Roche		AU 8931653
96		Roche		AU 8931653
97		Roche		AU 8931653
98		Roche		AU 8931653
99		Roche		AU 8931653

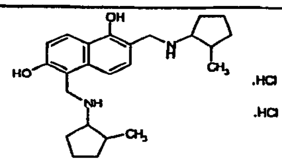
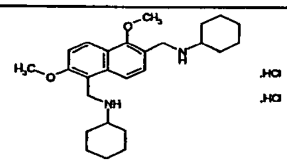
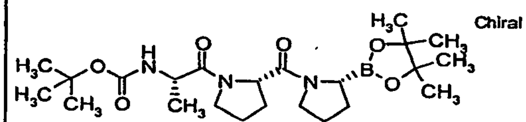
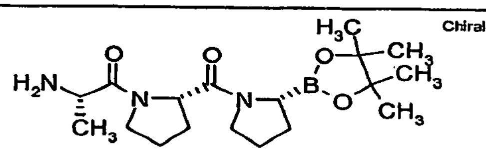
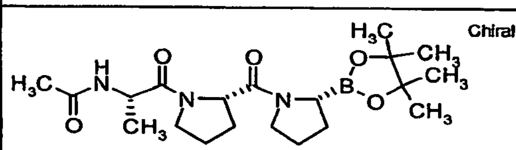
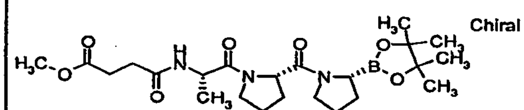
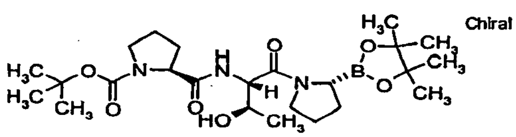
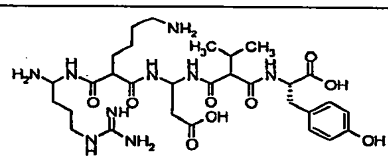
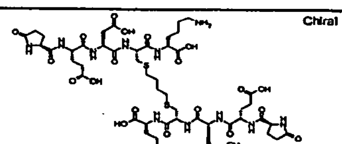
100		Roche		AU 8931653
101		Scharper	1) Migliorati, G. et al. 7th Int Cong Immunol (July 30-Aug 5, Berlin) 1989, Abst 106-62 .	
102		Mitsubishi Pharma		EP 351435
103		Abbott GmbH		EP 354693
104		Abbott GmbH		EP 354694
105		Wyeth		EP 354303
106		Merck & Co.		US 4866035
107		Abbott GmbH		EP 354693
108		Abbott GmbH		EP 354693

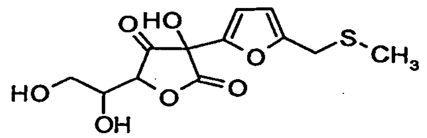
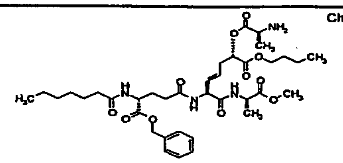
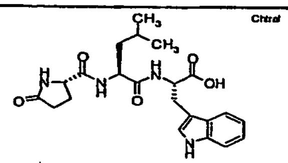
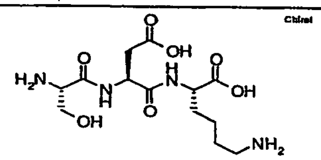
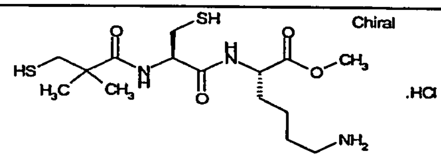
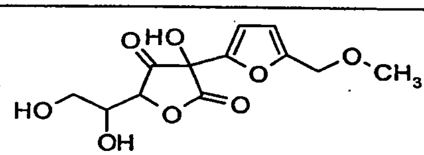
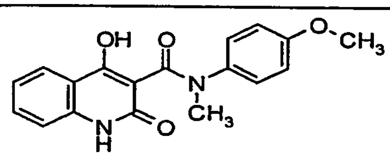
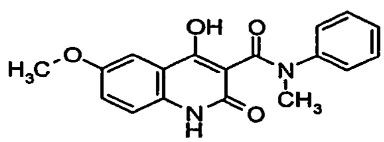
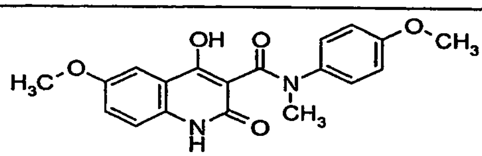
109		Abbott GmbH		EP 354693
110		Abbott GmbH		EP 354694
111		Abbott GmbH		EP 354694
112		Wyeth		EP 354303
113		Wyeth		EP 354303
114		Wyeth		EP 354303
115		Wyeth		EP 354303
116		Wyeth		EP 354303
117		Wyeth		EP 354303

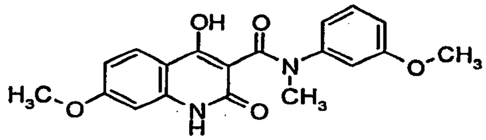
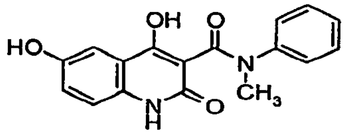
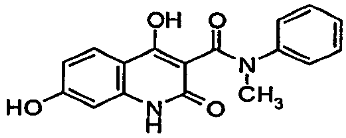
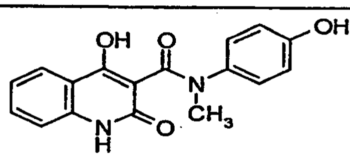
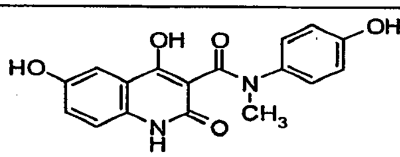
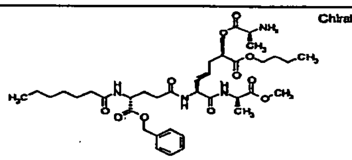
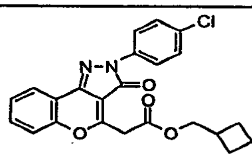
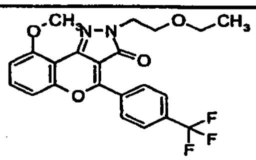
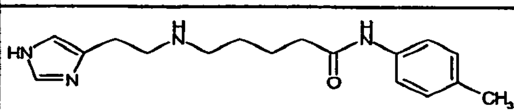
118		Wyeth		EP 354303
119		Wyeth		EP 354303
120		Wyeth		EP 354303
121		Wyeth		EP 354303
122		Wyeth		EP 354303
123		Wyeth		EP 354303
124		Wyeth		EP 354303
125		Wyeth		EP 354303
126		Wyeth		EP 354303

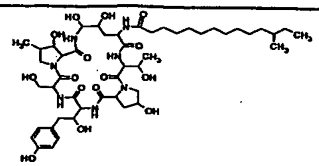
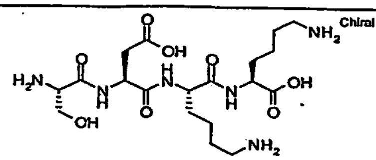
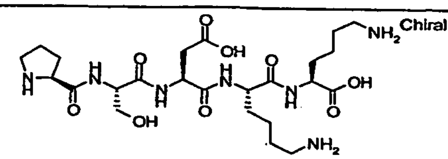
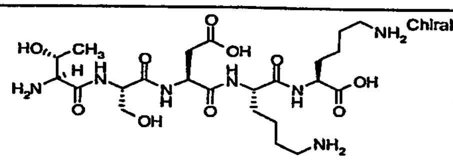
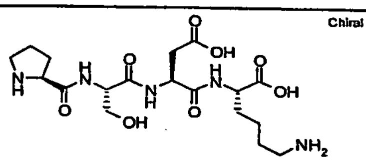
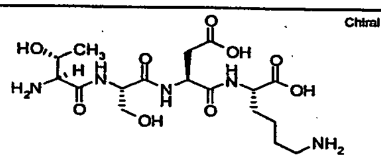
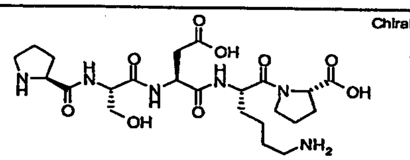
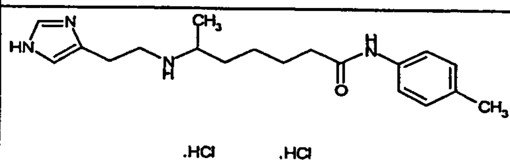
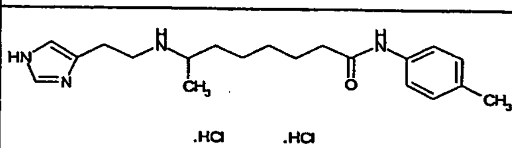
127		Wyeth		EP 354303
128		Tanabe		EP 372818
129		Elan	1) Eldon, M.A. et al. J Clin Pharmacol 1990, 30(4): 352-7.	
130		Lipha		EP 341104
131		Tanabe		EP 372818
132		Tanabe		EP 372818
133		Tanabe		EP 372818
134		Tanabe		EP 372818
135		Tanabe		EP 372818

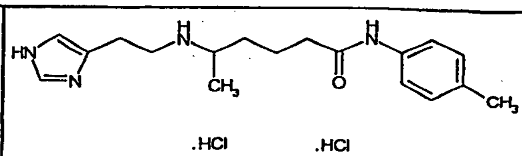
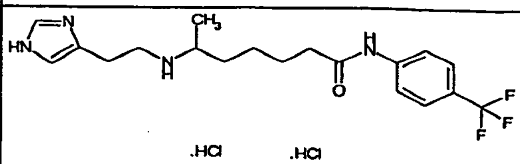
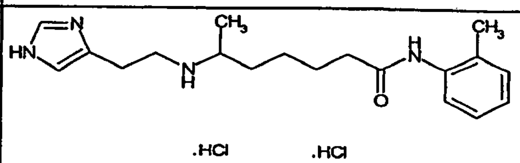
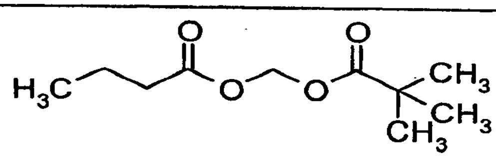
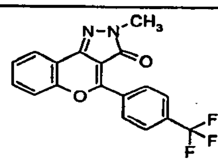
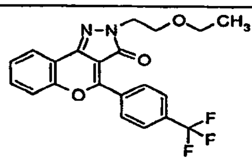
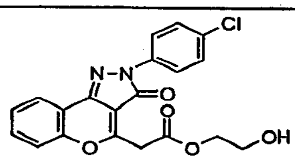
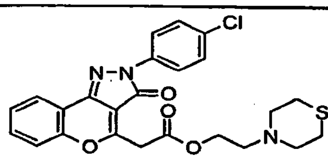
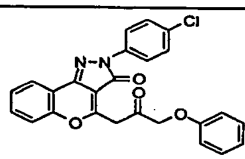
136	 Chiral	Tanabe		EP 372818
137	 Chiral	Tanabe		EP 372818
138	 Chiral	Tanabe		EP 372818
139	 Chiral	Tanabe		EP 372818
140	 Chiral	Bristol-Myers Squibb		US 4935493
141		Takeda		JP 90193940
142		Pharmacia		EP 429627
143		Merck & Co.		EP 393256
144		Merck & Co.		EP 393256

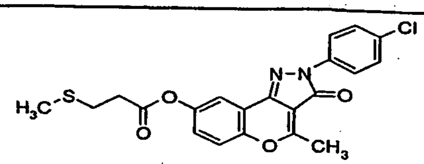
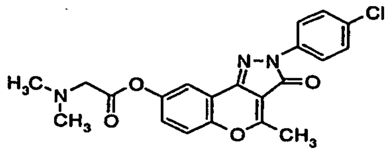
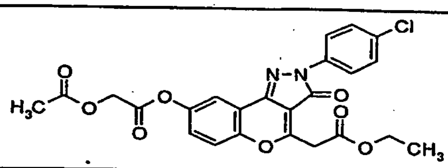
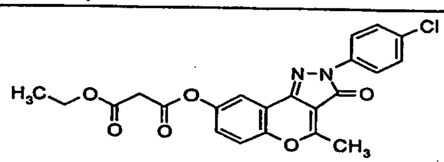
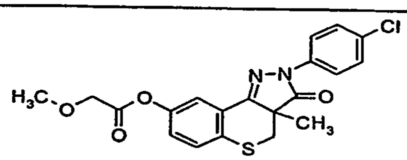
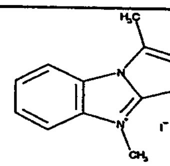
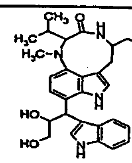
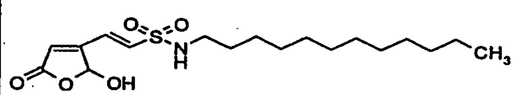
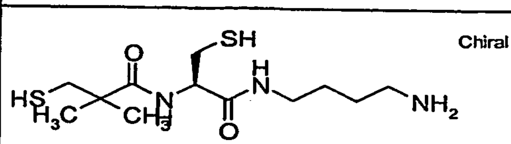
145		Merck & Co.		EP 393256
146		Merck & Co.		EP 393256
147		Bristol-Myers Squibb		US 4935493
148		Bristol-Myers Squibb		US 4935493
149		Bristol-Myers Squibb		US 4935493
150		Bristol-Myers Squibb		US 4935493
151		Bristol-Myers Squibb		US 4935493
152		Sclavo		EP 375058
153		SmithKline Beecham		AU 9059014

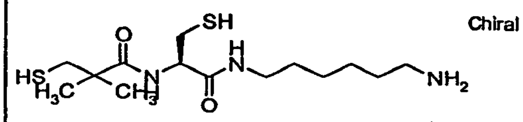
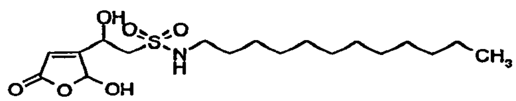
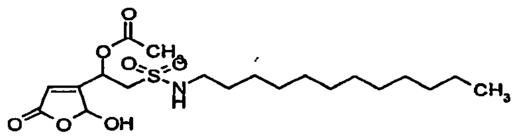
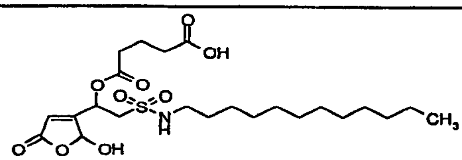
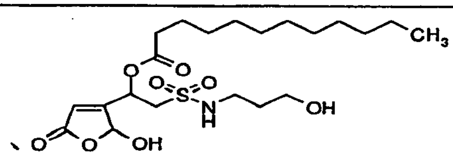
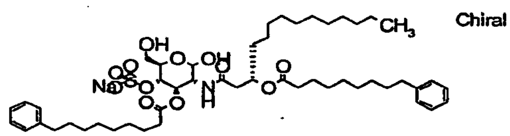
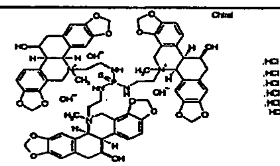
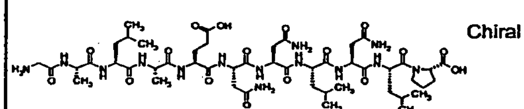
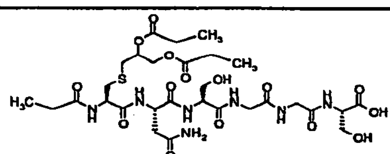
154		American Biotechnology		WO 9102733
155		Novartis		EP 417803
156		Polifarma		EP 423077
157		SCRAS		GB 2228937
158		Santen		EP 455833
159		American Biotechnology		WO 9102733
160		Pharmacia		EP 429627
161		Pharmacia		EP 429627
162		Pharmacia		EP 429627

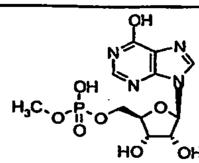
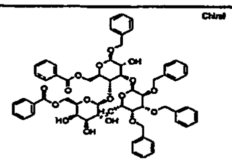
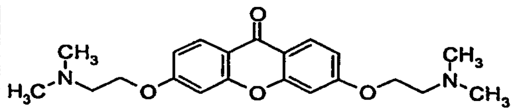
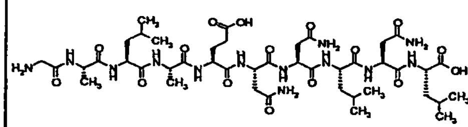
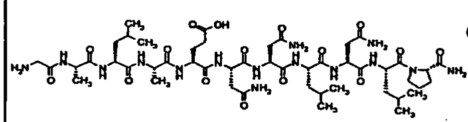
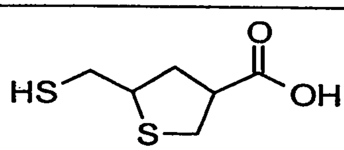
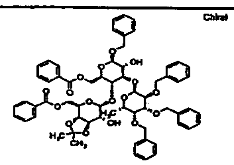
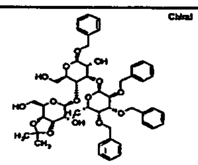
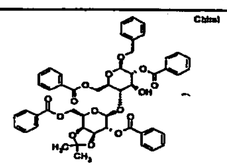
163		Pharmacia		EP 429627
164		Pharmacia		EP 429627
165		Pharmacia		EP 429627
166		Pharmacia		EP 429627
167		Pharmacia		EP 429627
168		Novartis		EP 417803
169		Abbott GmbH		WO 9111448
170		Abbott GmbH		WO 9112255
171	 .HCl .HCl	Leland Stanford Junior University		US 4996221

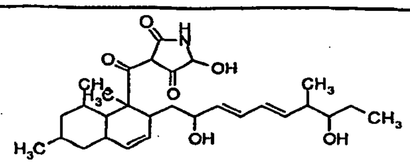
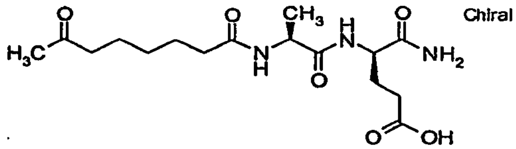
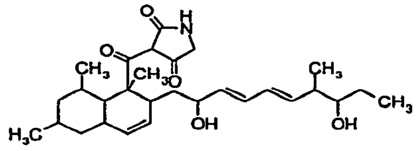
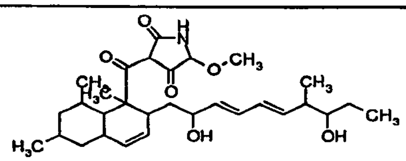
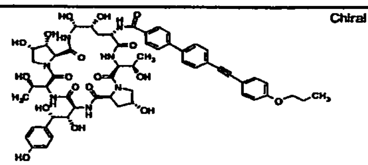
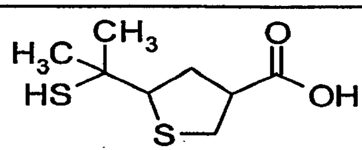
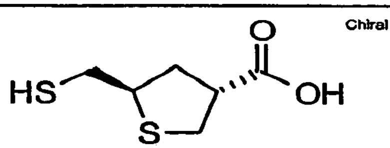
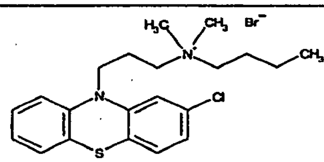
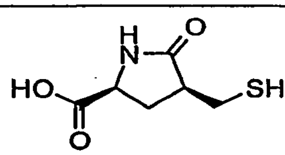
172		Aventis Pharma		EP 438813
173		SCRAS		GB 2228937
174		SCRAS		GB 2228937
175		SCRAS		GB 2228937
176		SCRAS		GB 2228937
177		SCRAS		GB 2228937
178		SCRAS		GB 2228937
179		Leland Stanford Junior University		US 4996221
180		Leland Stanford Junior University		US 4996221

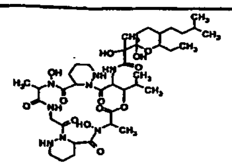
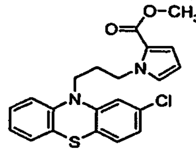
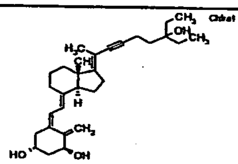
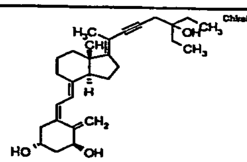
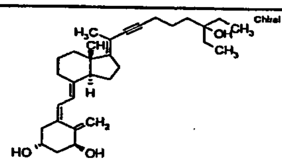
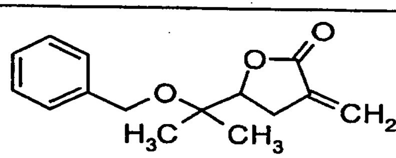
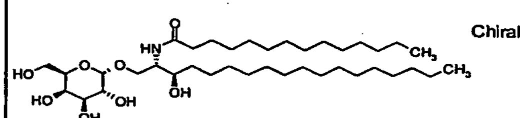
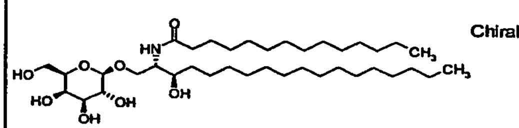
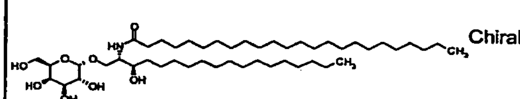
181		Leland Stanford Junior University		US 4996221
182		Leland Stanford Junior University		US 4996221
183		Leland Stanford Junior University		US 4996221
184		Bar-Ilan University	1) Rephaeli, A. et al. Int J Cancer 1991, 49(1): 66.	EP 302349
185		Abbott GmbH		WO 9112255
186		Abbott GmbH		WO 9112255
187		Abbott GmbH		WO 9111448
188		Abbott GmbH		WO 9111448
189		Abbott GmbH		WO 9111448

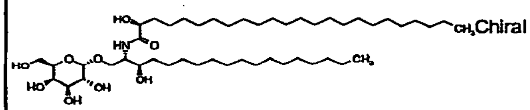
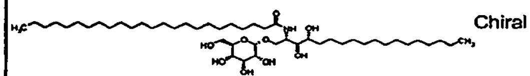
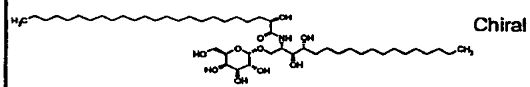
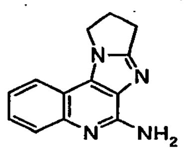
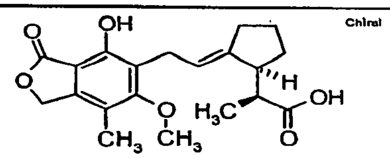
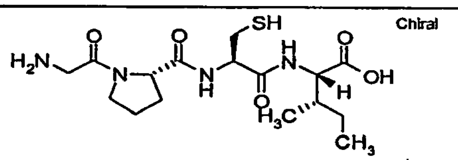
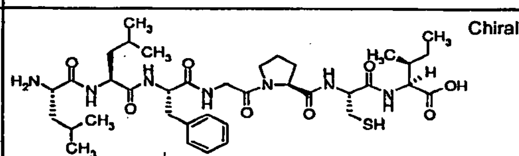
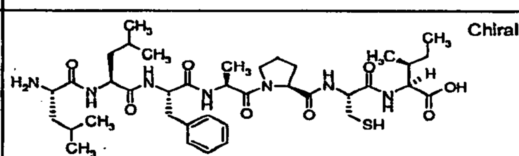
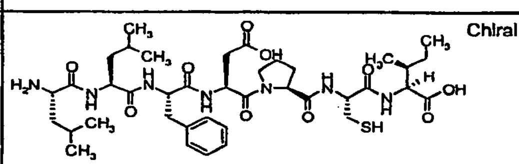
190		Abbott GmbH		WO 9111448
191		Abbott GmbH		WO 9111448
192		Abbott GmbH		WO 9111448
193		Abbott GmbH		WO 9111448
194		Abbott GmbH		WO 9111448
195		Bashkir Medical University	Dianov, V.M. et al. Khim Farm Zh SSSR 1991, 25(1): 40.	
196		Microbial Chemistry Research Foundation	Kumagai, H. et al. J Antibiot 1991, 44(9): 1029.	
197		Allergan		US 5081261
198		Santen		EP 455833

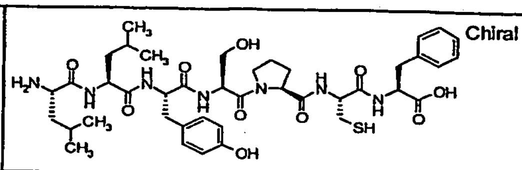
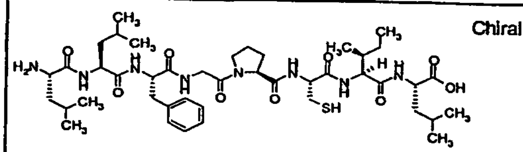
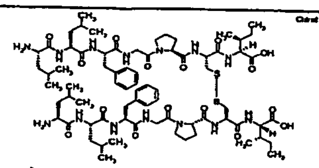
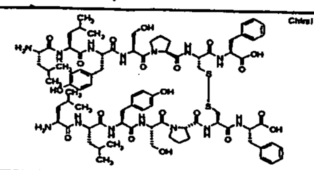
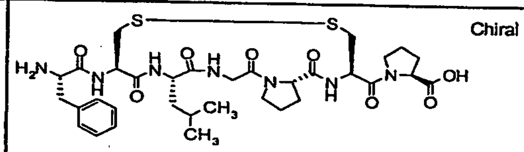
199		Santen		EP 455833
200		Allergan		US 5081261
201		Allergan		US 5081261
202		Allergan		US 5081261
203		Allergan		US 5081261
204		Ono	1) Satoh, M. et al. 51st Annu Meet Jpn Cancer Assoc (Sept 29-Oct 1, Osaka) 1992, Abst 1450 .	EP 553786
205		Nowicky	1) Hohenwarter, O.;Strutzenberger, K.;Katinger, H.;Liepins, A.;Nowicky, J.W. Drug Exp Clin Res 1992, 18(Suppl.): 1.	WO 8300486
206		Tanabe		US 5210075
207		Fujisawa	1) Kurimura, M. et al. Pept Chem (1991) 1992, 361.	

208		University of South Florida	1) Hadden, J.W. 5th Interscl World Conf Inflamm, Antirheum, Analg, Immunomodul (April 25-28, Geneva) 1993, Abst 257 .	
209		Glycomed		WO 9310796
210		Karolinska Institute	1) Algarra, I. et al. Int J Cancer 1993, 54: 518.	
211	 Chiral	Tanabe		US 5210075
212	 Chiral	Tanabe		US 5210075
213		Santen		EP 558287
214		Glycomed		WO 9310796
215		Glycomed		WO 9310796
216		Glycomed		WO 9310796

217		Microbial Chemistry Research Foundation		
218		LEK	1) Sersa, G. et al. Mol Biother 1992, 4: 188.	EP 477912
219		Microbial Chemistry Research Foundation	1) Ueno, M. et al. J Antibiot 1993, 46(5): 719.	
220		Microbial Chemistry Research Foundation		
221		Lilly	1) Bartlett, M.S. et al. 33rd Intersci Conf Antimicrob Agents Chemother (Oct 17-20, New Orleans) 1993, Abst 369 .	EP 561639
222		Santen		EP 558287
223		Santen		EP 558287
224		ADIR		EP 572308
225		Santen		JP 93239045

226		Microbial Chemistry Research Foundation	Ueno, M. et al. J Antibiot 1993, 46(11): 1658.	
227		ADIR		EP 572308
228		Leo		WO 9401398
229		Leo		WO 9401398
230		Leo		WO 9401398
231		Santen		JP 93239045
232		Kirin Brewery	1) Morita, M. et al. J Med Chem 1995, 38(12): 2176.	1) EP 609437
233		Kirin Brewery		1) EP 609437
234		Kirin Brewery		1) EP 609437

235		Kirin Brewery		EP 609437
236		Kirin Brewery		EP 609437
237		Kirin Brewery		EP 609437
238		3M Pharmaceuticals	Lindstrom, K.J. et al. 211th ACS Natl Meet (March 24-28, New Orleans) 1996, Abst MEDI 210 .	US 5482936
239		Roche Bioscience	Smith, D.B. et al. J Org Chem 1996, 61(6): 2236.	WO 9522538
240		AstraZeneca		WO 9611943
241		AstraZeneca		WO 9611943
242		AstraZeneca		WO 9611943
243		AstraZeneca		WO 9611943

244		AstraZeneca		WO 9611943
245		AstraZeneca		WO 9611943
246		AstraZeneca		WO 9611943
247		AstraZeneca		WO 9611943
248		AstraZeneca		WO 9611943
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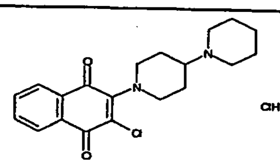
250		Japan Tobacco		JP 96113555
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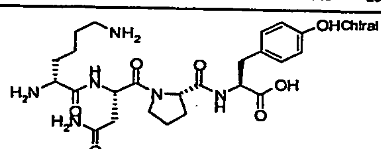
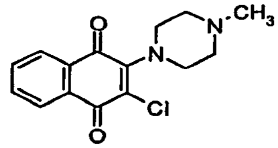
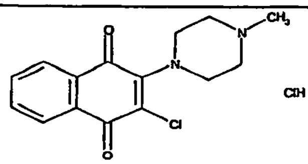
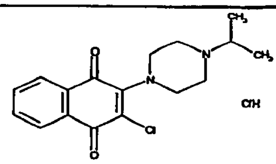
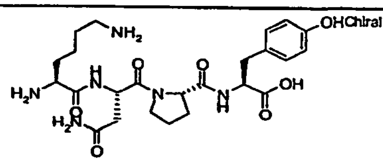
252		Shire BioChem		WO 9619494

253		Japan Tobacco		JP 96113555
254		Japan Tobacco		JP 96113555
255		Japan Tobacco		JP 96113555
256		Shire BioChem		WO 9619494
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260	Pro Lys Pro Pro Lys Pro Ala Met Arg Met Lys Ser Val Thr Pro Leu Leu Met Glu Ala Met Pro Leu	Harvard College		JP 96504177
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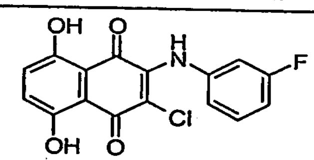
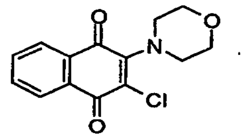
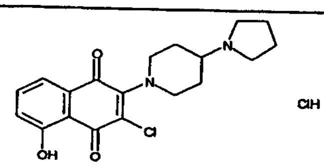
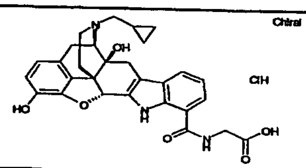
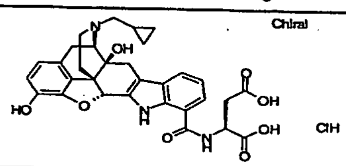
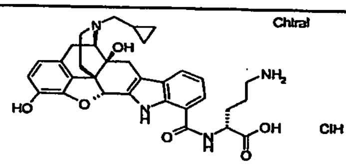
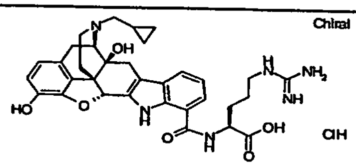
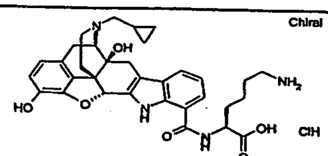
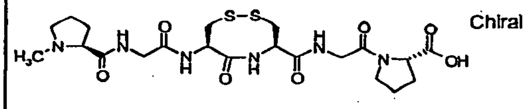
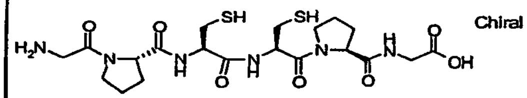
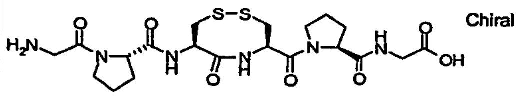
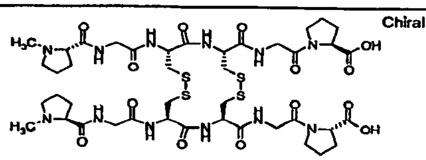
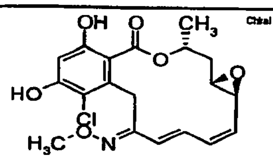
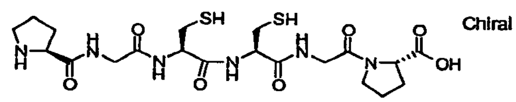
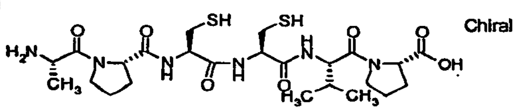
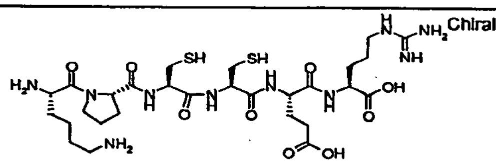
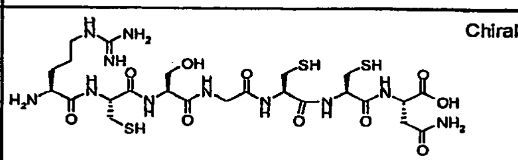
263		Japan Tobacco		JP 96113555
264		Japan Tobacco		JP 96113555
265		Japan Tobacco		JP 96113555
266		Univ. Minnesota		WO 9623793
267		Univ. Minnesota		WO 9623793
268		Univ. Minnesota		WO 9623793
269		Univ. Minnesota		WO 9623793
270		Univ. Minnesota		WO 9623793

271		AstraZeneca		WO 9630397
272		AstraZeneca		WO 9630397
273		AstraZeneca		WO 9630397
274		AstraZeneca		WO 9630397
275		Kyowa Hakko		WO 9633989
276		AstraZeneca		WO 9630397
277		AstraZeneca		WO 9630397
278		AstraZeneca		WO 9630397
279		AstraZeneca		WO 9630397

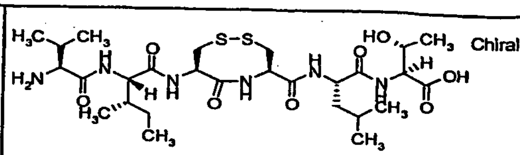
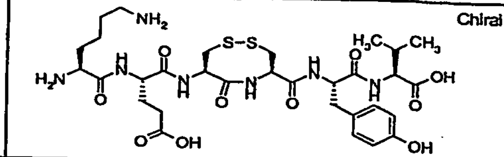
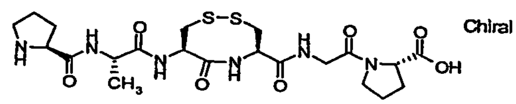
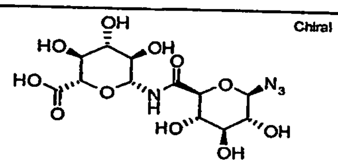
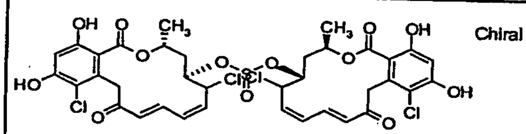
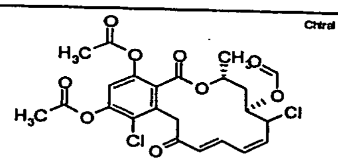
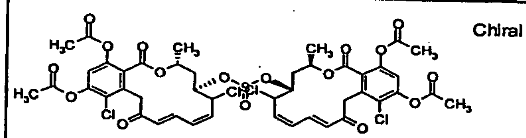
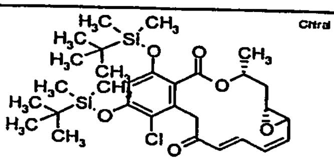
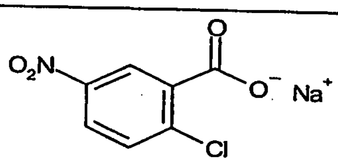
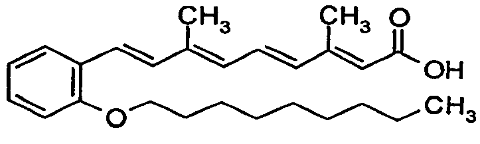
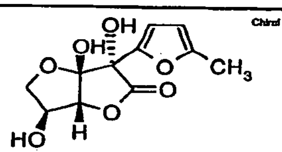
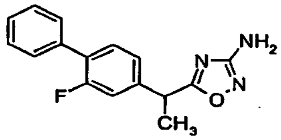
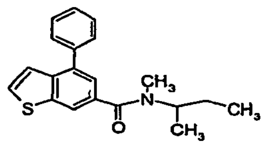
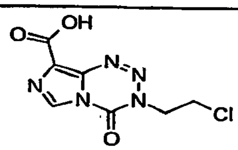
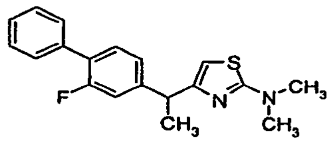
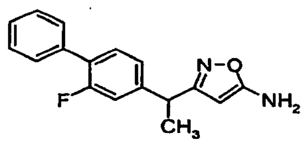
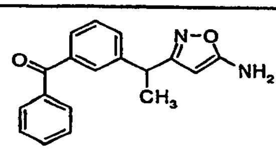
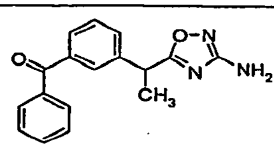
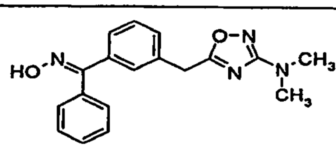
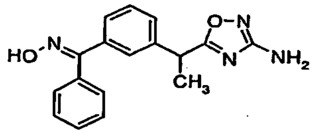
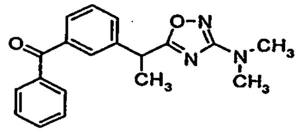
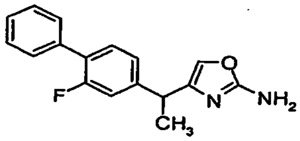
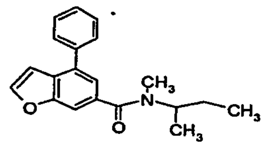
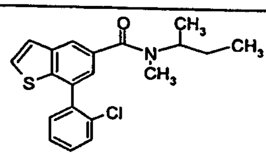
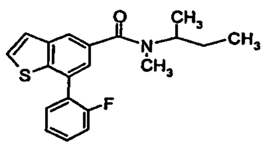
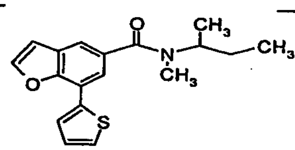
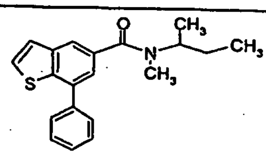
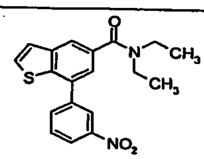
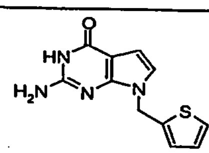
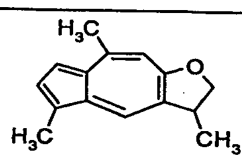
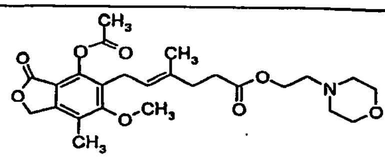
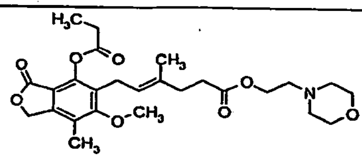
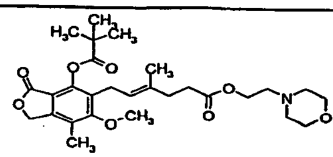
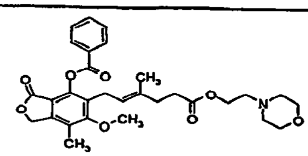
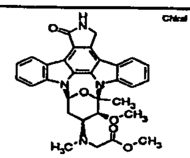
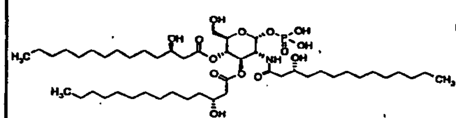
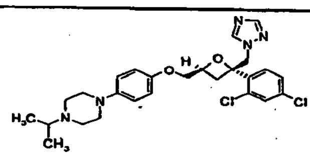
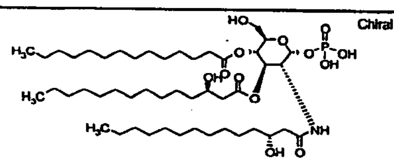
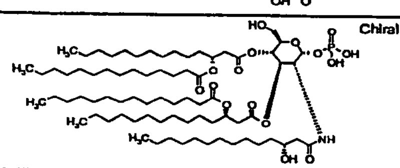
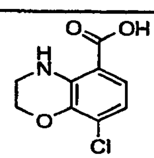
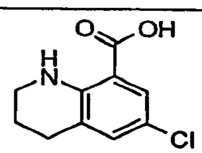
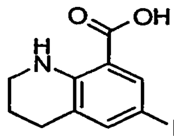
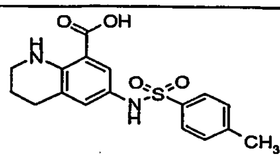
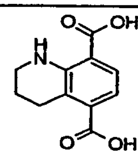
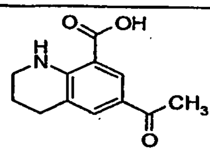
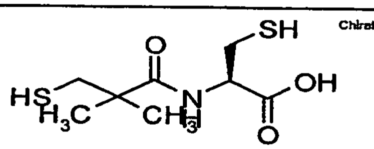
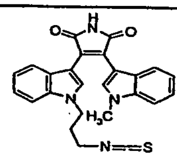
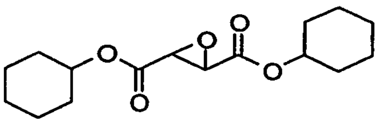
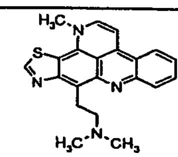
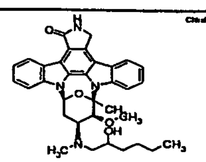
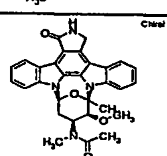
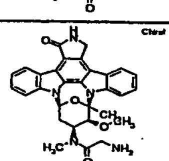
280		AstraZeneca		WO 9630397
281		AstraZeneca		WO 9630397
282		AstraZeneca		WO 9630397
283		Glycomed		WO 9635700
284		Kyowa Hakko		WO 9633989
285		Kyowa Hakko		WO 9633989
286		Kyowa Hakko		WO 9633989
287		Kyowa Hakko		WO 9633989
288		Aston University	Kinchington, D. et al. 4th Conf Retroviruses Opportunistic Infect (Jan 22-26, Washington DC) 1997, Abst .	

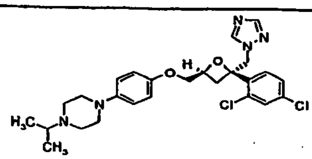
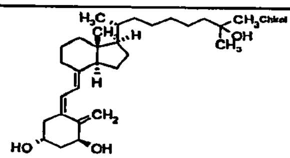
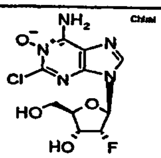
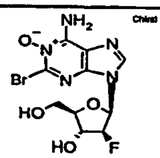
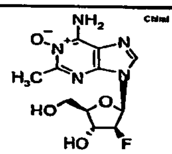
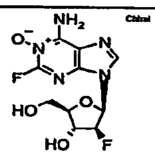
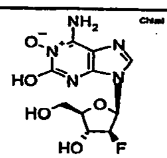
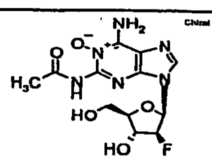
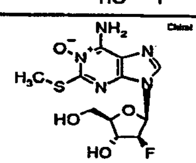
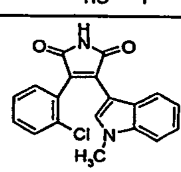
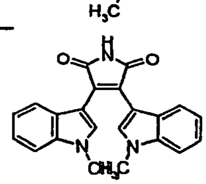
Table 35 Continued

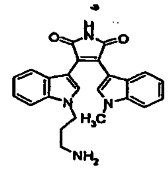
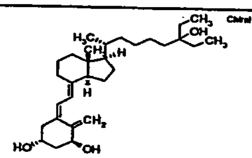
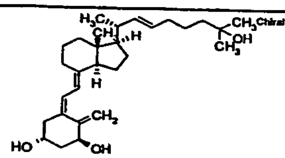
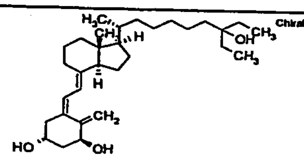
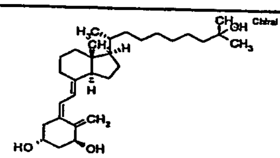
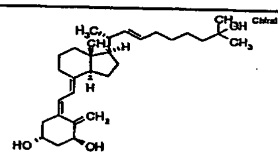
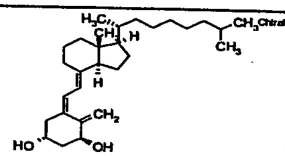
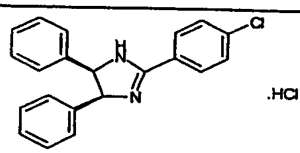
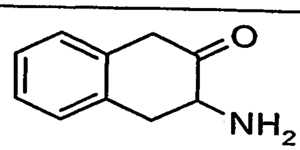
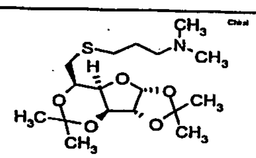
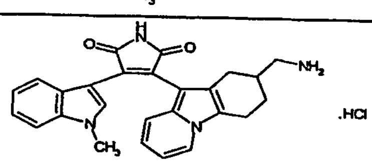
Compound #	Structure	Source	Literature Reference	Patent Number
289		Roche	1) Fiedler-Nagy, C. et al. Agent Action 1989, 27(3-4): 313-5.	EP 169571
290		NFCR	1) Forest Laboratories, Inc. Annual Report 1994.	WO 9517890
291		Sumitomo Pharmaceuticals		EP 248399
292		Aventis Pharma		EP 248734
293		May & Baker		EP 252682
294		Sumitomo Pharmaceuticals		EP 248399
295		Sumitomo Pharmaceuticals		EP 248399
296		Sumitomo Pharmaceuticals		EP 248399
297		Sumitomo Pharmaceuticals		EP 248399
298		Sumitomo Pharmaceuticals		EP 248399

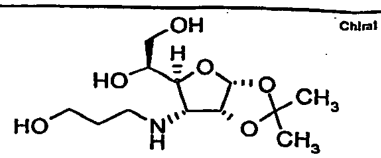
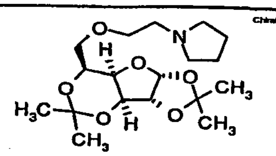
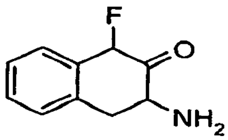
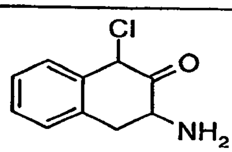
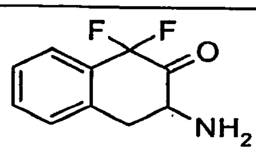
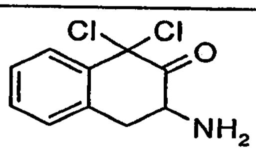
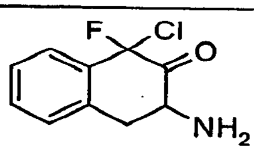
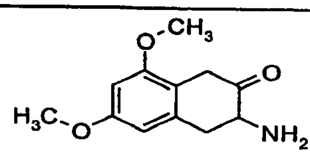
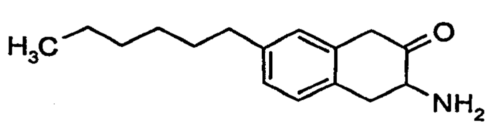
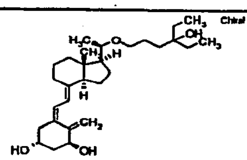
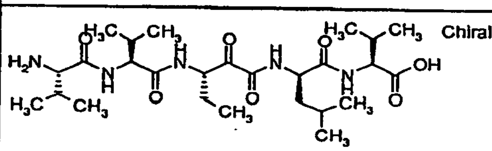
299		Sumitomo Pharmaceuticals		EP 248399
300		Sumitomo Pharmaceuticals		EP 248399
301		Sumitomo Pharmaceuticals		EP 248399
302		Aventis Pharma		EP 248734
303		Aventis Pharma		EP 248734
304		Aventis Pharma		EP 248734
305		Aventis Pharma		EP 248734
306		Aventis Pharma		EP 248734
307		Aventis Pharma		EP 248734
308		Pfizer		AU 8783281
309		Harbor Branch Found.		US 4755529

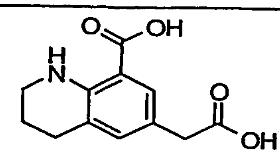
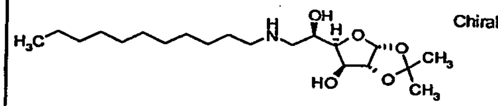
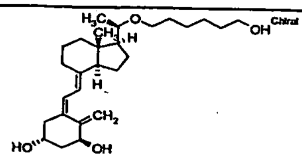
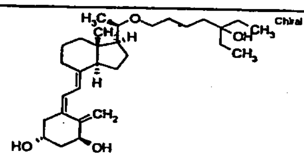
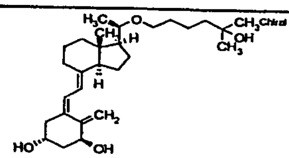
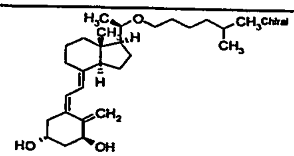
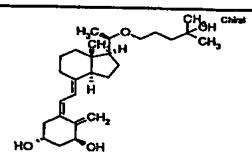
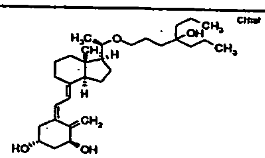
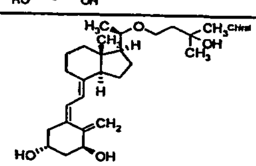
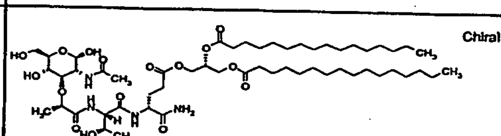
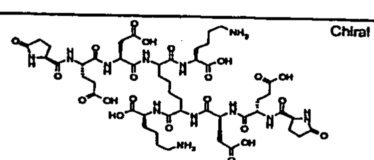
310		Roche Bioscience		AU 8782540
311		Roche Bioscience		AU 8782540
312		Roche Bioscience		AU 8782540
313		Roche Bioscience		AU 8782540
314		Novartis		EP 296110
315	 Chiral	Novartis	1) Lam, C. et al. Antimicrob Agents Chemother 1991, 35(3): 500.	AU 8822785
316		Schering-Plough		EP 318214
317		Novartis		AU 8822785
318		Novartis		AU 8822785
319		Kyorin		EP 310096
320		Kyorin		EP 310096

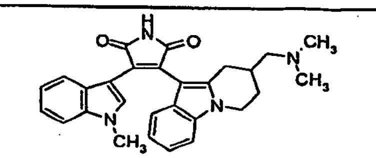
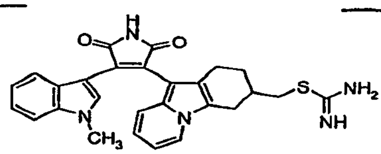
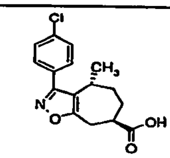
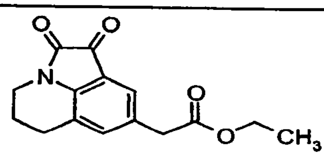
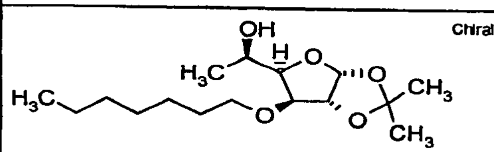
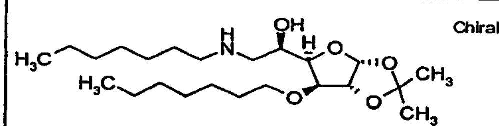
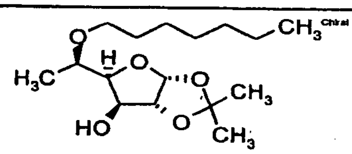
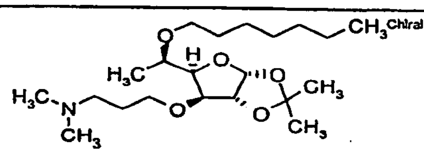
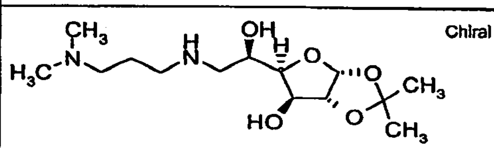
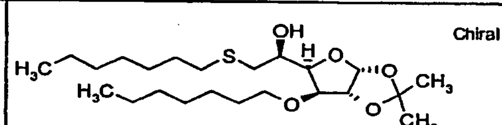
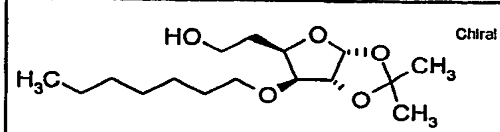
321		Kyorin		EP 310096
322		Kyorin		EP 310096
323		Kyorin		EP 310096
324		Kyorin		EP 310096
325		Santen		EP 326326
326		Roche		AU 8929658
327		Taisho	1) Takeshita, K. et al. Int J Immunother 1988, 4(2): 97-106.	JP 79141750
328		Harbor Branch Found.	1) Burres, N.S. et al. Proc Amer Assoc Cancer Res 1989, 30: Abst 1914.	EP 331320
329		Novartis		EP 296110
330		Novartis		EP 296110
331		Novartis		EP 296110

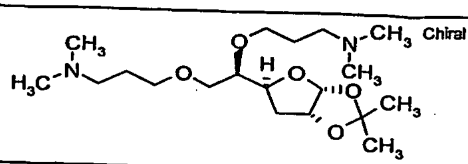
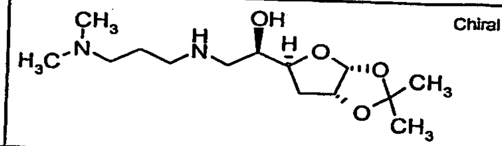
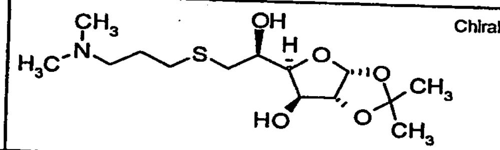
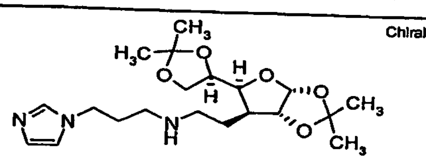
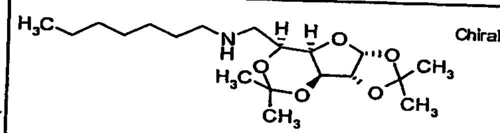
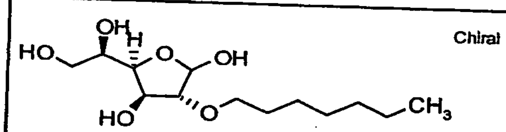
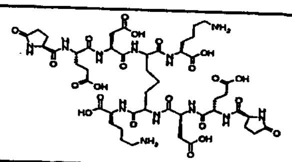
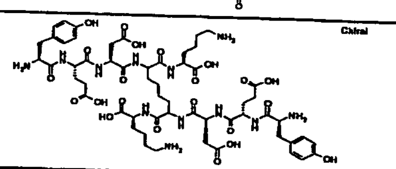
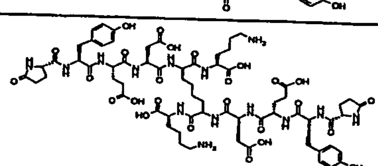
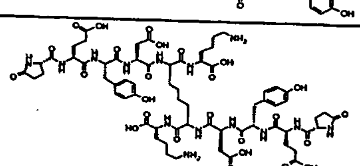
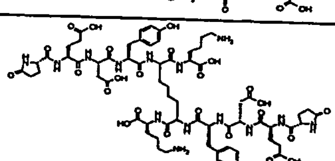
332		Schering-Plough		EP 318214
333		Leo		WO 8910351
334		Scripps Clinic Res. Found.		WO 8908658
335		Scripps Clinic Res. Found.		WO 8908658
336		Scripps Clinic Res. Found.		WO 8908658
337		Scripps Clinic Res. Found.		WO 8908658
338		Scripps Clinic Res. Found.		WO 8908658
339		Scripps Clinic Res. Found.		WO 8908658
340		Scripps Clinic Res. Found.		WO 8908658
341		Roche		AU 8929658
342		Roche		AU 8929658

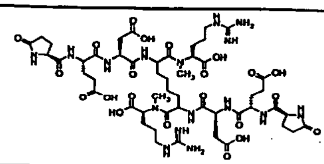
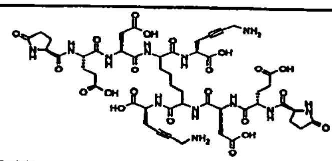
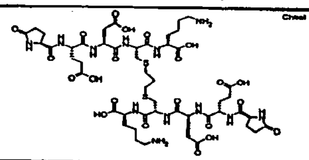
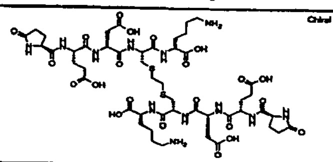
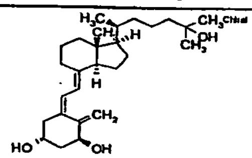
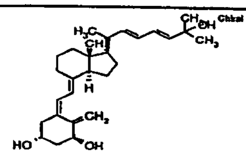
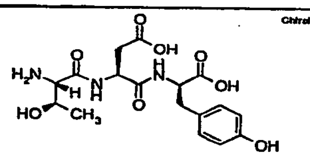
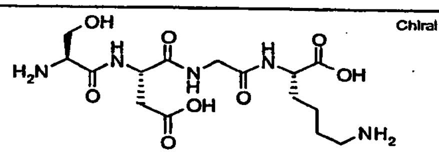
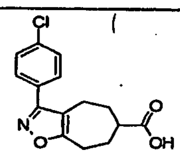
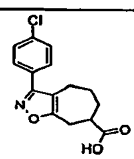
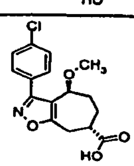
343		Roche		AU 8929658
344		Leo		WO 8910351
345		Leo		WO 8910351
346		Leo		WO 8910351
347		Leo		WO 8910351
348		Leo		WO 8910351
349		Leo		WO 8910351
350		Tanabe Seiyaku	1) Ueno, M. et al. Jpn J Pharmacol 1992, 58(Suppl. 1): Abst O-210.	AU 8942368
351		Aventis Pharma		EP 378456
352		Greenwich Pharm.		AU 9047648
353		Roche		EP 384349

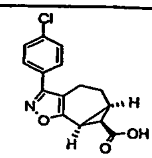
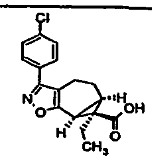
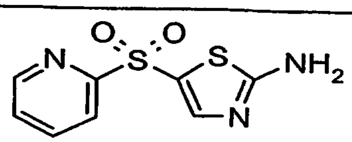
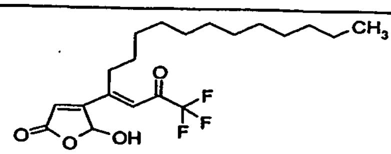
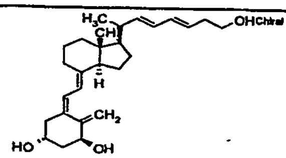
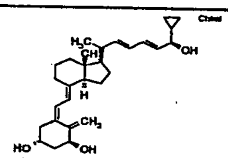
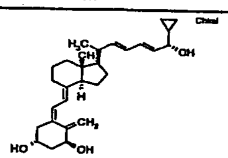
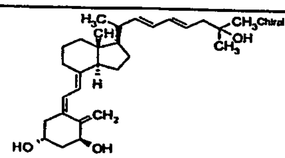
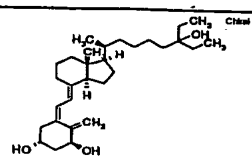
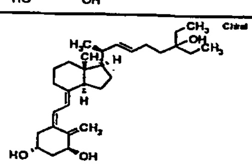
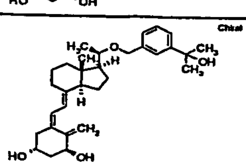
354		Greenwich Pharm.		AU 9047648
355		Greenwich Pharm.		AU 9047648
356		Aventis Pharma		EP 378456
357		Aventis Pharma		EP 378456
358		Aventis Pharma		EP 378456
359		Aventis Pharma		EP 378456
360		Aventis Pharma		EP 378456
361		Aventis Pharma		EP 378456
362		Aventis Pharma		EP 378456
363		Leo	Binderup, L. et al. Biochem Pharmacol 1991, 42(8): 1569.	EP 460032
364		Microbial Chemistry Research Foundation	Muraoka, Y. et al. 30th Intersci Conf Antimicrob Agents Chemother (Oct 21-24, Atlanta) 1990, Abst 801 .	

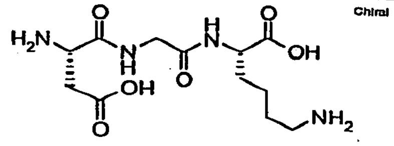
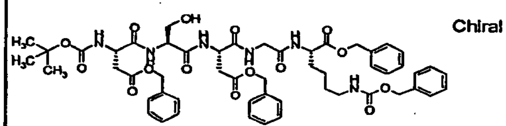
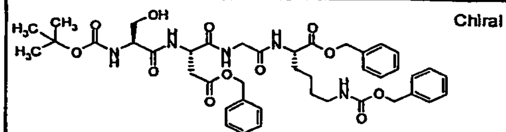
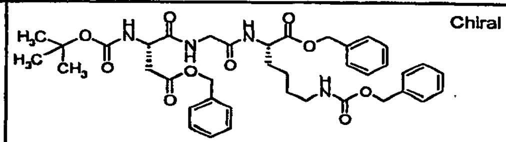
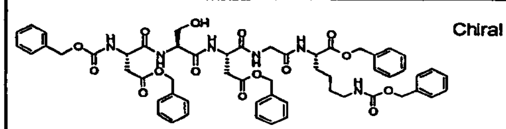
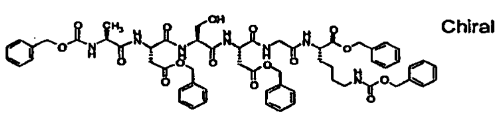
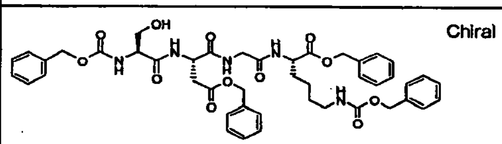
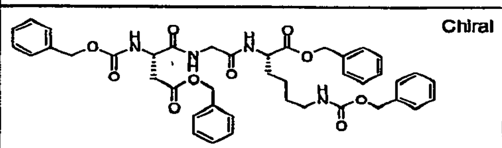
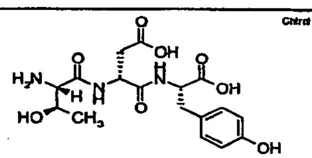
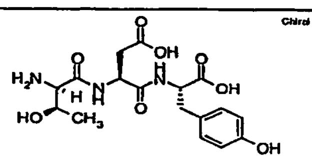
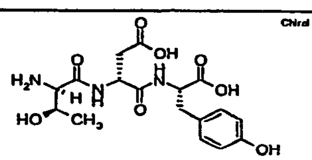
365		Kyorin		AU 9057029
366		Greenwich Pharm.		AU 9057691
367		Leo		EP 460032
368		Leo		EP 460032
369		Leo		EP 460032
370		Leo		EP 460032
371		Leo		EP 460032
372		Leo		EP 460032
373		Leo		EP 460032
374		Novartis	Kricek, F. et al. Immunopharmacology 1997, 36(1): 27.	AU 9057875
375		Amersham Health	1) Frey, C.L. et al. 31st Intersci Conf Antimicrob Agents Chemother (Sept 29-Oct 2, Chicago) 1991, Abst 85 .	AU 9059014

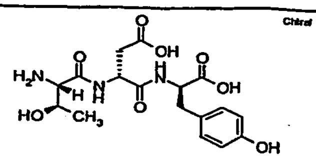
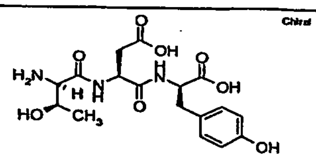
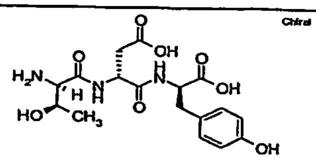
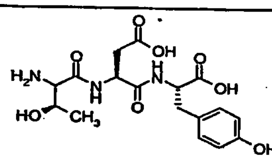
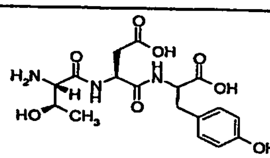
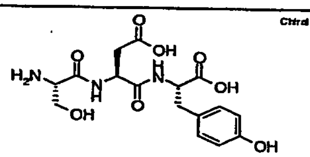
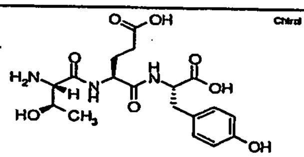
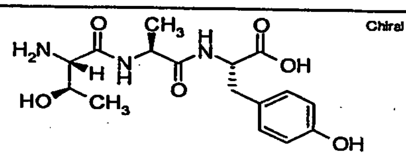
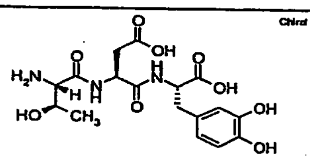
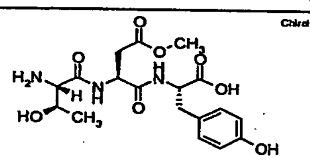
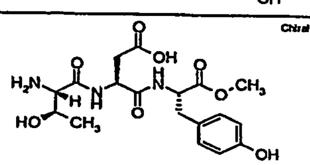
376		Roche		EP 384349
377		Roche		EP 384349
378		Roche		AU 9059154
379		Kyorin		AU 9057029
380		Greenwich Pharm.		AU 9057691
381		Greenwich Pharm.		AU 9057691
382		Greenwich Pharm.		AU 9057691
383		Greenwich Pharm.		AU 9057691
384		Greenwich Pharm.		AU 9057691
385		Greenwich Pharm.		AU 9057691
386		Greenwich Pharm.		AU 9057691

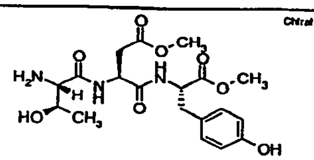
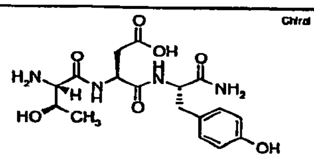
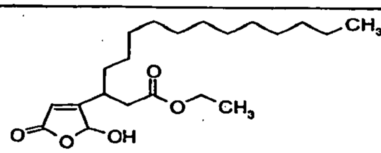
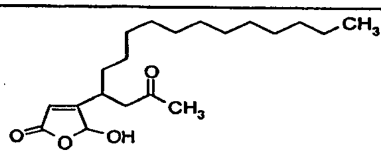
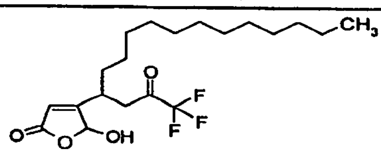
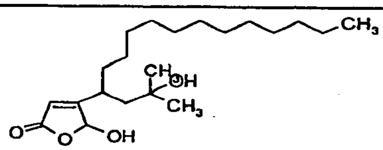
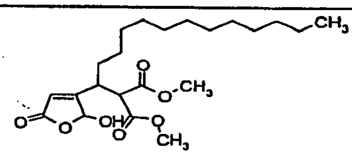
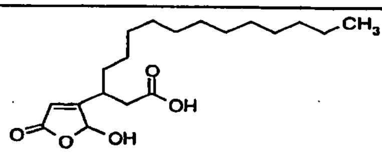
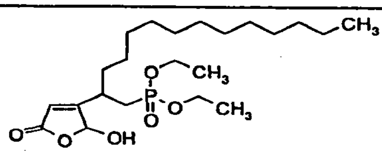
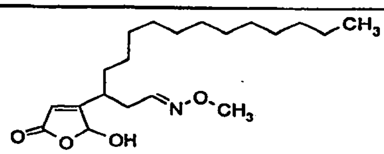
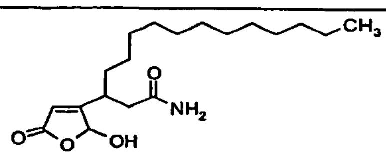
387		Greenwich Pharm.		AU 9057691
388		Greenwich Pharm.		AU 9057691
389		Greenwich Pharm.		AU 9057691
390		Greenwich Pharm.		AU 9057691
391		Greenwich Pharm.		AU 9057691
392		Greenwich Pharm.		AU 9057691
393		Amersham Health		AU 9059014
394		Amersham Health		AU 9059014
395		Amersham Health		AU 9059014
396		Amersham Health		AU 9059014
397		Amersham Health		AU 9059014

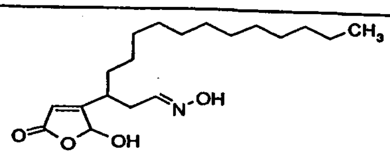
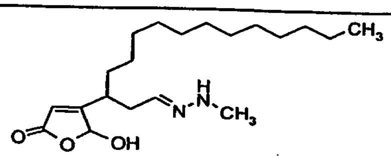
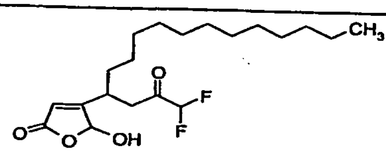
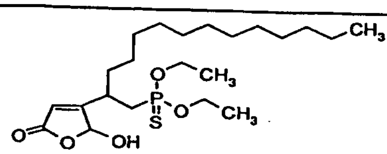
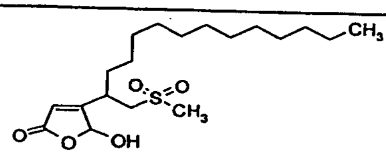
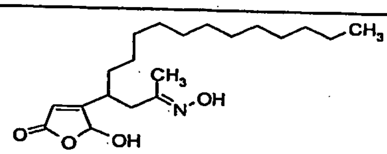
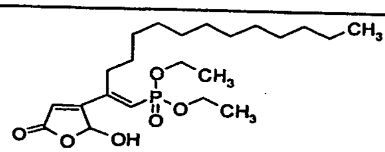
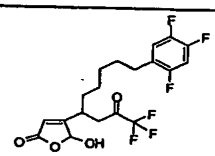
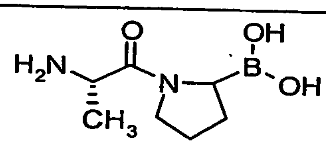
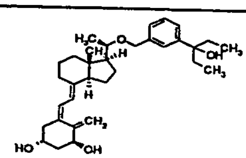
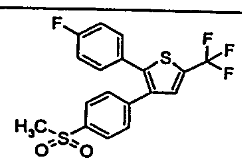
398		Amersham Health		AU 9059014
399		Amersham Health		AU 9059014
400		Amersham Health		AU 9059014
401		Amersham Health		AU 9059014
402		Leo	1) Elstner, E. et al. Blood 1994, 84(6): 1960.	EP 479871
403		Leo		WO 9100855
404		SPA		EP 421074
405		Hitachi Chemical		EP 421682
406		Roche		AU 9059154
407		Roche		AU 9059154
408		Roche		AU 9059154

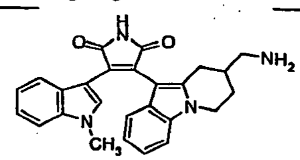
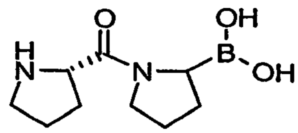
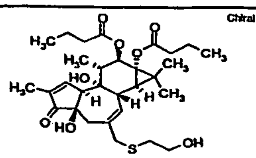
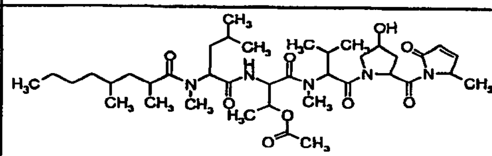
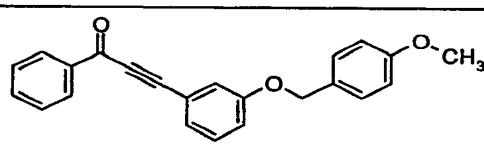
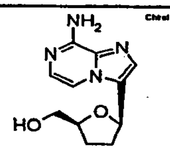
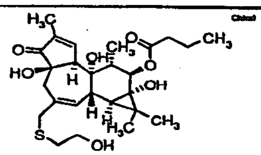
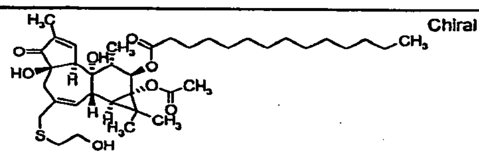
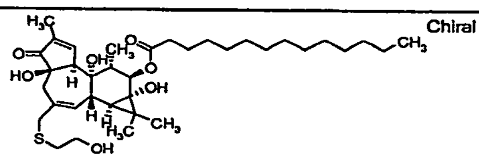
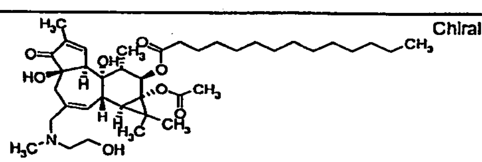
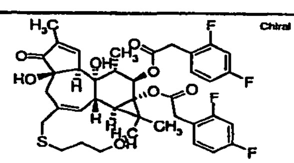
409		Roche		AU 9059154
410		Roche		AU 9059154
411		Fujisawa	1) Manda, T. et al. Jpn J Pharmacol 1997, 73(Suppl. 1): Abst P-140.	EP 412404
412		Allergan		US 5013850
413		Leo		WO 9100855
414		Leo		WO 9100855
415		Leo		WO 9100855
416		Leo		WO 9100855
417		Leo		EP 479871
418		Leo		EP 479871
419		Leo		WO 9109841

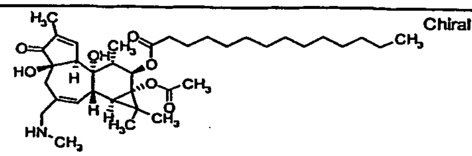
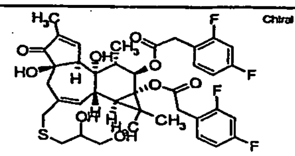
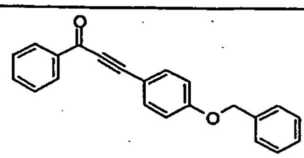
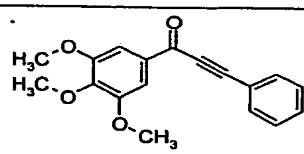
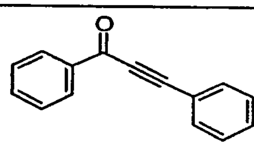
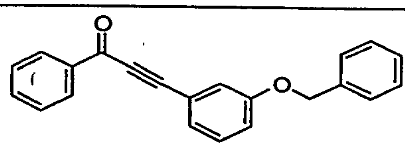
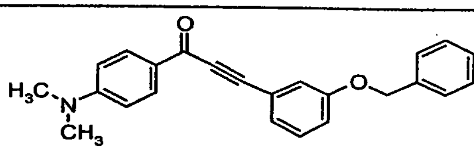
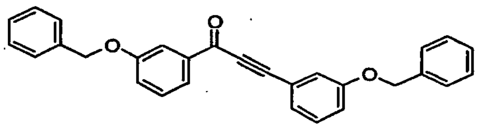
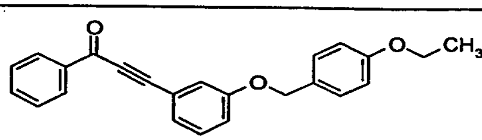
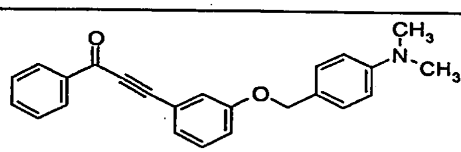
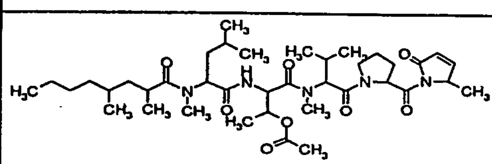
420		Hitachi Chemical		EP 421682
421		Hitachi Chemical		EP 421682
422		Hitachi Chemical		EP 421682
423		Hitachi Chemical		EP 421682
424		Hitachi Chemical		EP 421682
425		Hitachi Chemical		EP 421682
426		Hitachi Chemical		EP 421682
427		Hitachi Chemical		EP 421682
428		SPA		EP 421074
429		SPA		EP 421074
430		SPA		EP 421074

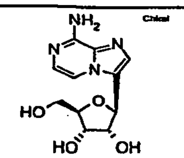
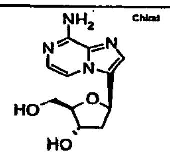
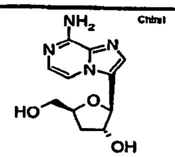
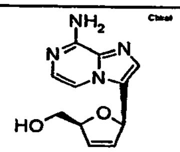
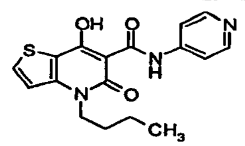
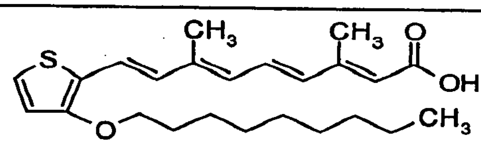
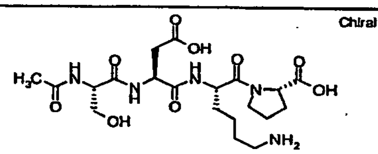
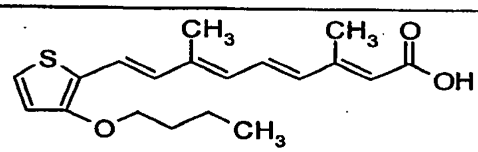
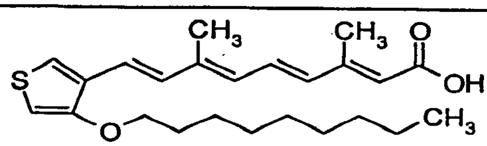
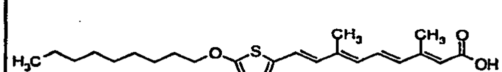
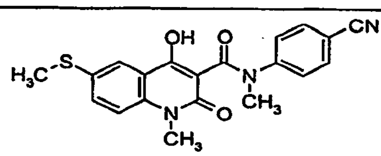
431		SPA		EP 421074
432		SPA		EP 421074
433		SPA		EP 421074
434		SPA		EP 421074
435		SPA		EP 421074
436		SPA		EP 421074
437		SPA		EP 421074
438		SPA		EP 421074
439		SPA		EP 421074
440		SPA		EP 421074
441		SPA		EP 421074

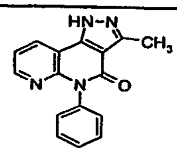
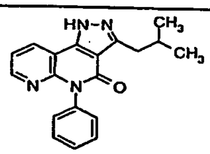
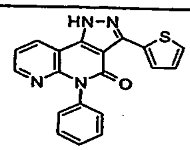
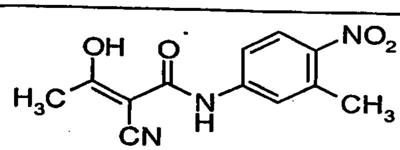
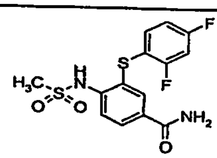
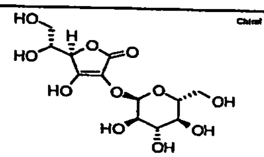
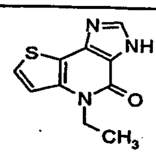
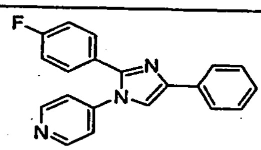
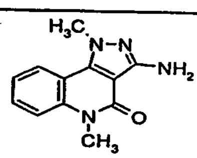
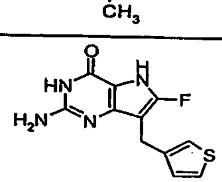
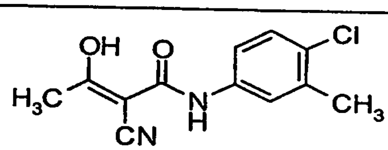
442		SPA		EP 421074
443		SPA		EP 421074
444		Allergan		US 5013850
445		Allergan		US 5013850
446		Allergan		US 5013850
447		Allergan		US 5013850
448		Allergan		US 5013850
449		Allergan		US 5013850
450		Allergan		US 5013850
451		Allergan		US 5013850
452		Allergan		US 5013850

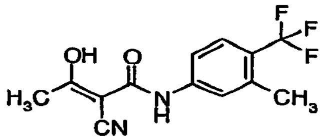
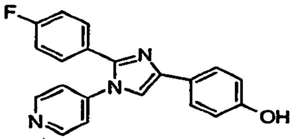
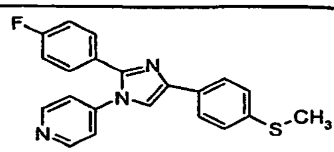
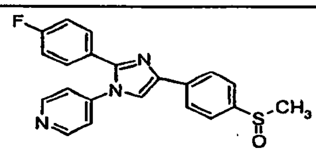
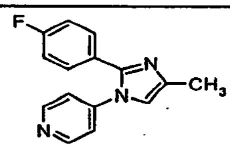
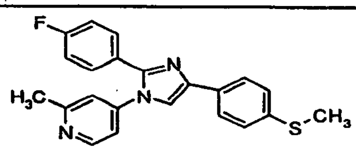
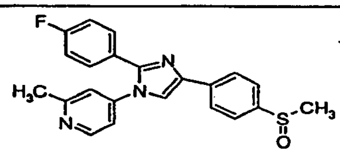
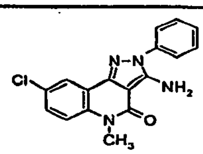
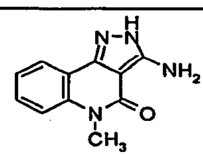
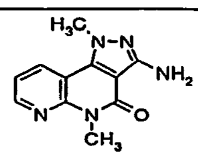
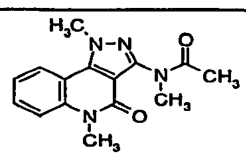
453		Allergan		US 5013850
454		Allergan		US 5013850
455		Allergan		US 5013850
456		Allergan		US 5013850
457		Allergan		US 5013850
458		Allergan		US 5013850
459		Allergan		US 5013850
460		Allergan		US 5013850
461		New England Med. Center Hosp.		WO 9116339
462		Leo		EP 506794
463		Fujisawa		WO 9119708

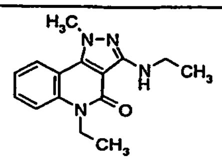
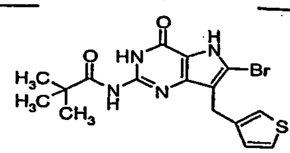
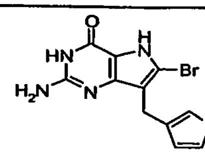
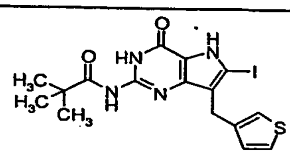
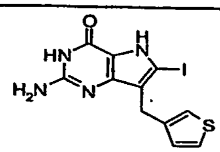
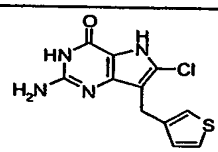
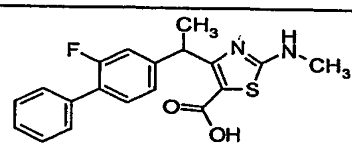
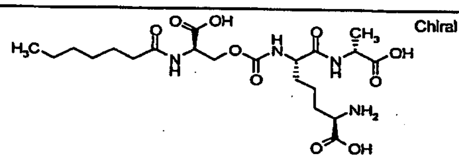
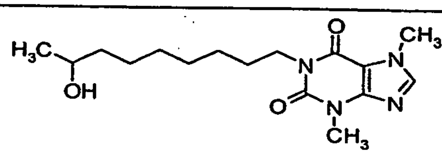
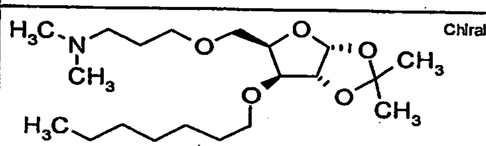
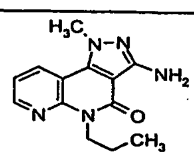
464		Roche	1) Hill, C.H. 6th SCI-RSC Med Chem Symp (Sept 8-11, Cambridge) 1991, Abst S18 .	EP 384349
465		New England Med. Center Hosp.		WO 9116339
466		Alder		US 5145842
467		Harbor Branch Found.		US 5091368
468		Aventis Pharma		EP 476658
469		Merck & Co.		EP 480713
470		Alder		US 5145842
471		Alder		US 5145842
472		Alder		US 5145842
473		Alder		US 5145842
474		Alder		US 5145842

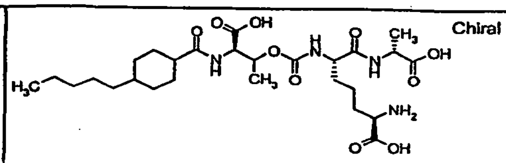
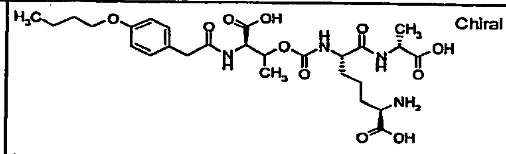
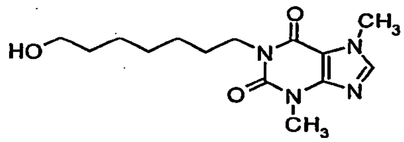
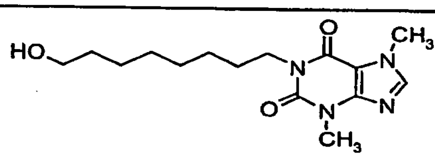
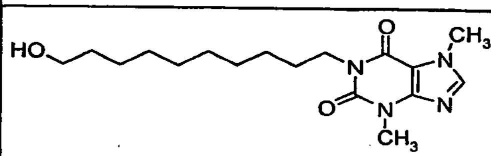
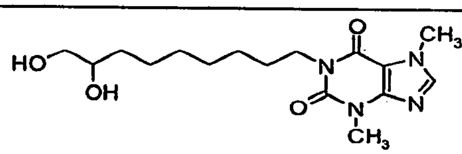
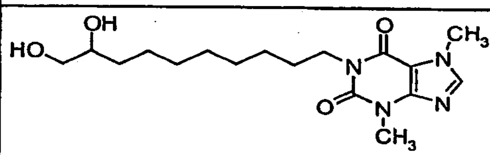
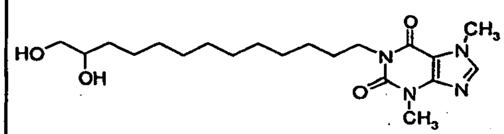
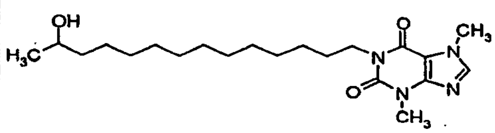
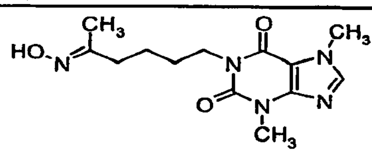
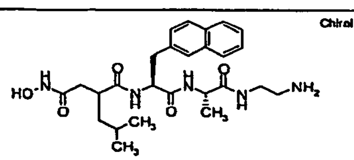
475		Alder		US 5145842
476		Alder		US 5145842
477		Aventis Pharma		EP 476658
478		Aventis Pharma		EP 476658
479		Aventis Pharma		EP 476658
480		Aventis Pharma		EP 476658
481		Aventis Pharma		EP 476658
482		Aventis Pharma		EP 476658
483		Aventis Pharma		EP 476658
484		Aventis Pharma		EP 476658
485		Harbor Branch Found.		US 5091368

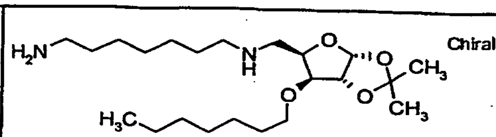
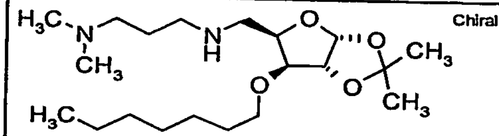
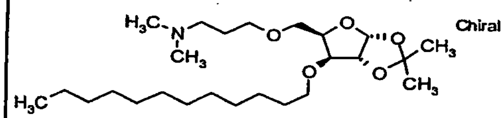
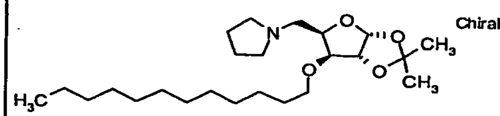
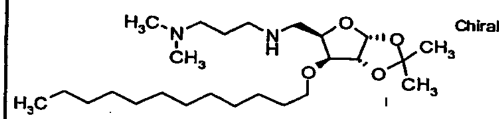
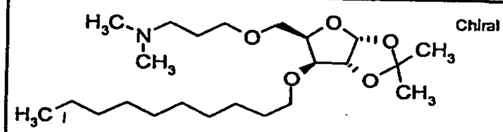
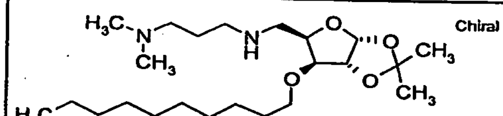
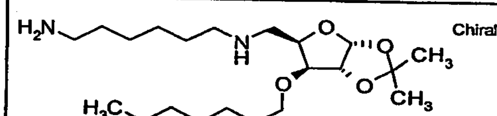
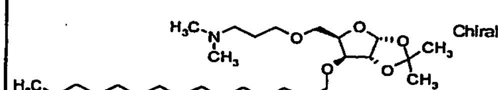
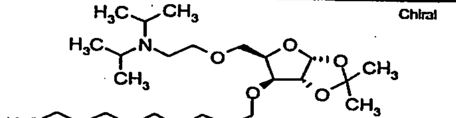
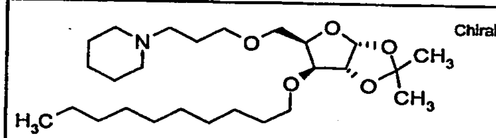
486		Merck & Co.		EP 480713
487		Merck & Co.		EP 480713
488		Merck & Co.		EP 480713
489		Merck & Co.		EP 480713
490		Kyowa Hakko	Miwa, K. et al. 113th Annu Meet Pharmaceut Soc Jpn (March 29-31, Osaka) 1993, Abst 30CC 13-1 .	EP 505058
491		Roche		EP 510473
492		Beaufour-Ipsen	1) Carde, P. et al. Proc Amer Soc Clin Oncol 1991, 10: Abst 324.	AU 8810261
493		Roche		EP 510473
494		Roche		EP 510473
495		Roche		EP 510473
496		Fujisawa		WO 9218483

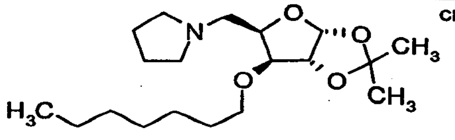
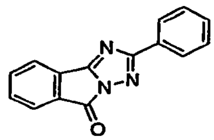
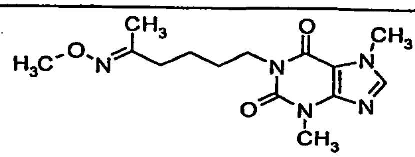
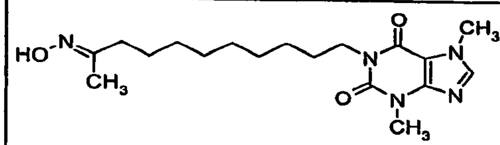
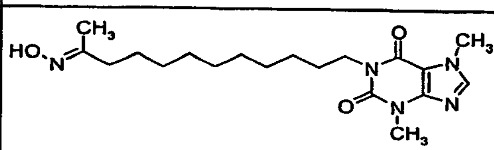
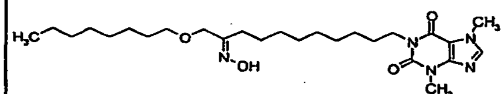
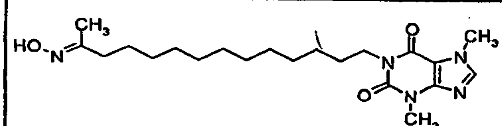
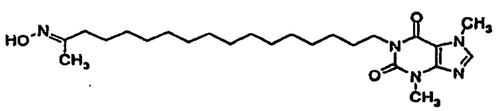
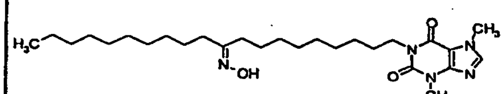
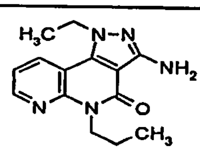
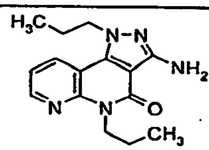
497		Kyowa Hakko		EP 526840
498		Kyowa Hakko		EP 526840
499		Kyowa Hakko		EP 526840
500		Aventis Pharma		EP 538783
501		Fujisawa	Nakamura, K. et al. Chem Pharm Bull 1993, 41(5): 894.	AU 8783152
502		Hayashibara	1) Yamamoto, I. et al. 18th Int Cong Chemother (June 27-July 2, Stockholm) 1993, Abst 516 .	EP 539196
503		Kyowa Hakko		WO 9312116
504		GlaxoSmithKline		WO 9314082
505		Otsuka		JP 93132484
506		Pfizer		US 5236926
507		Aventis Pharma		EP 538783

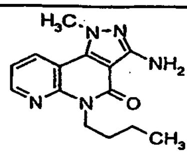
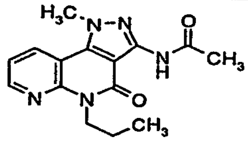
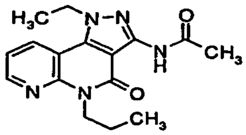
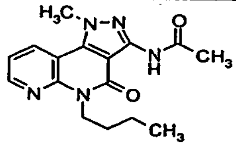
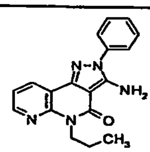
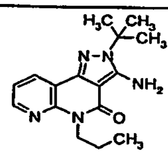
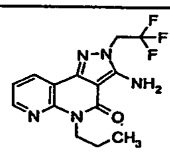
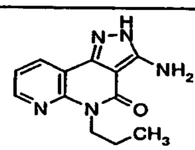
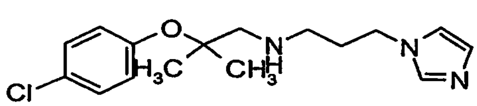
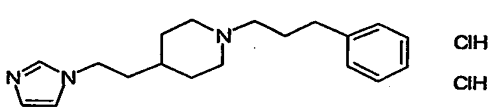
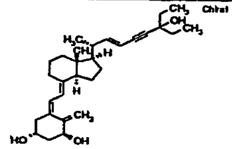
508		Aventis Pharma		EP 538783
509		GlaxoSmithKline		WO 9314082
510		GlaxoSmithKline		WO 9314082
511		GlaxoSmithKline		WO 9314082
512		GlaxoSmithKline		WO 9314082
513		GlaxoSmithKline		WO 9314082
514		GlaxoSmithKline		WO 9314082
515		Otsuka		JP 93132484
516		Otsuka		JP 93132484
517		Otsuka		JP 93132484
518		Otsuka		JP 93132484

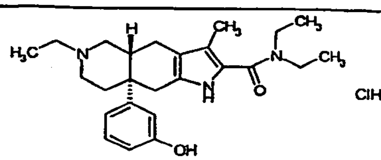
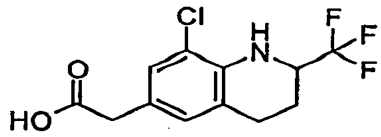
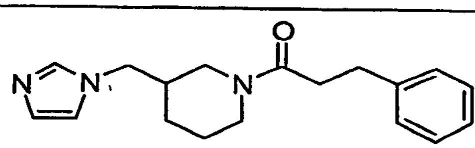
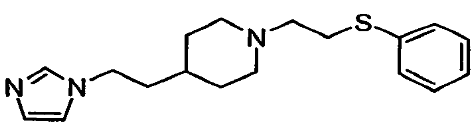
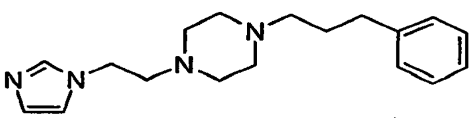
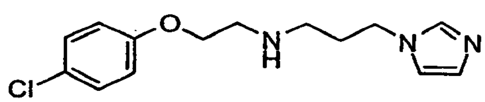
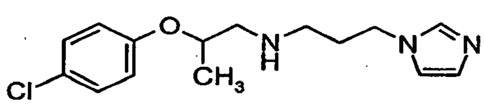
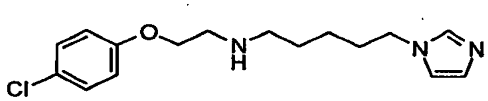
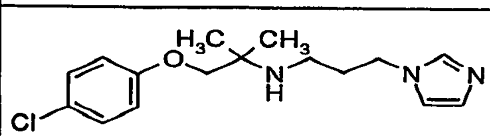
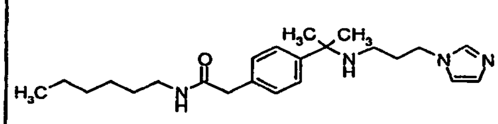
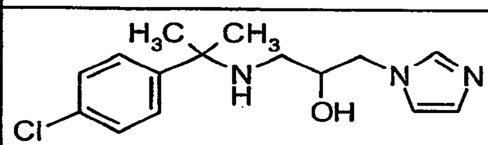
519		Otsuka		JP 93132484
520		Pfizer		US 5236926
521		Pfizer		US 5236926
522		Pfizer		US 5236926
523		Pfizer		US 5236926
524		Pfizer		US 5236926
525		Sumitomo		US 5258396
526		Wyeth		US 5312831
527		Cell Therapeutics		WO 9411001
528		Greenwich Pharm.		WO 9411381
529		Otsuka		JP 94100561

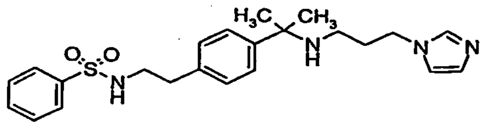
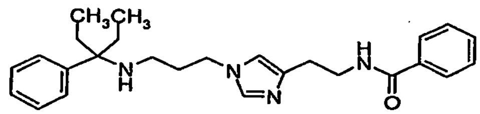
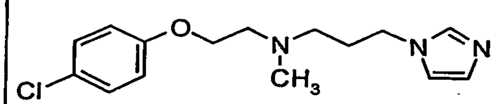
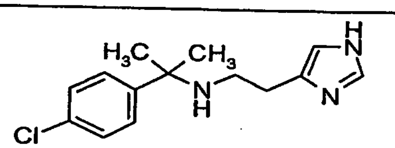
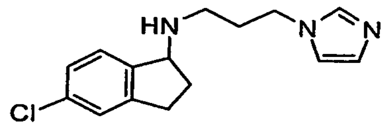
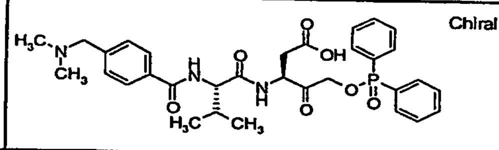
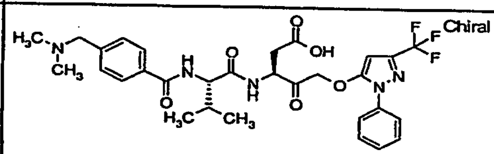
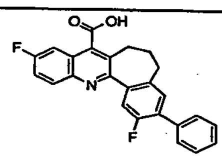
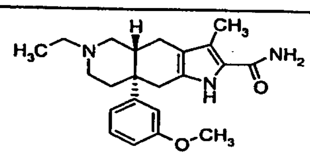
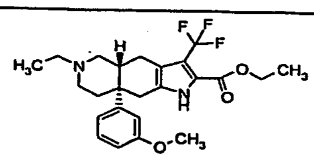
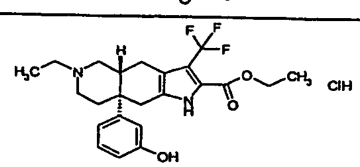
530		Wyeth		US 5312831
531		Wyeth		US 5312831
532		Cell Therapeutics		WO 9411001
533		Cell Therapeutics		WO 9411001
534		Cell Therapeutics		WO 9411001
535		Cell Therapeutics		WO 9411001
536		Cell Therapeutics		WO 9411001
537		Cell Therapeutics		WO 9411001
538		Cell Therapeutics		WO 9411001
539		Cell Therapeutics		WO 9416704
540		Immunex	1) Immunex Corporation Press Release 1994, July 21.	WO 9506031

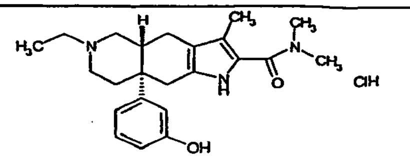
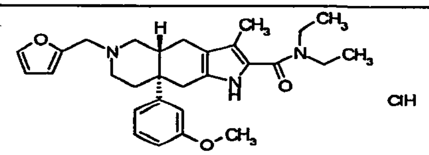
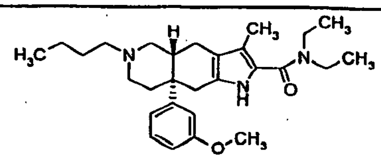
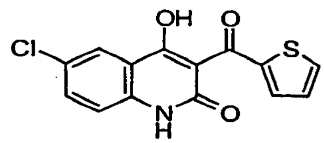
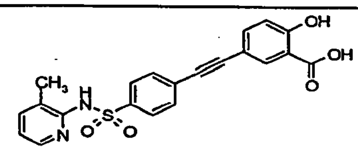
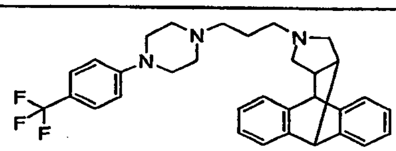
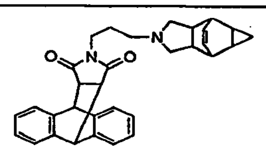
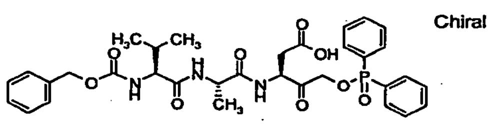
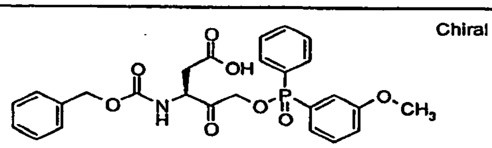
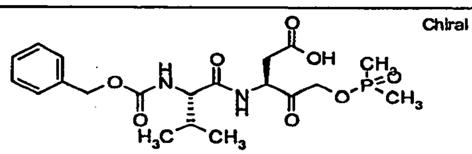
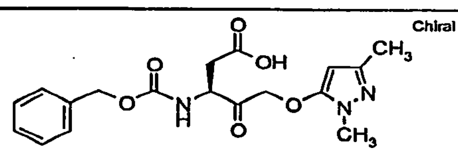
541		Greenwich Pharm.		WO 9411381
542		Greenwich Pharm.		WO 9411381
543		Greenwich Pharm.		WO 9411381
544		Greenwich Pharm.		WO 9411381
545		Greenwich Pharm.		WO 9411381
546		Greenwich Pharm.		WO 9411381
547		Greenwich Pharm.		WO 9411381
548		Greenwich Pharm.		WO 9411381
549		Greenwich Pharm.		WO 9411381
550		Greenwich Pharm.		WO 9411381
551		Greenwich Pharm.		WO 9411381

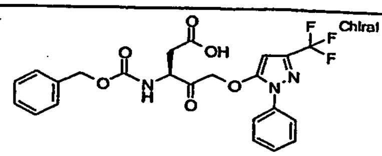
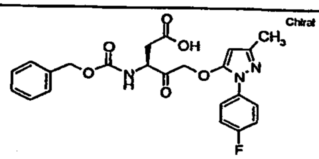
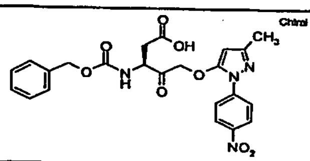
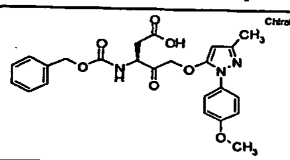
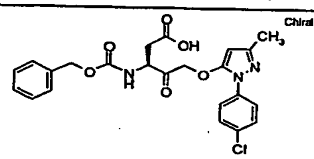
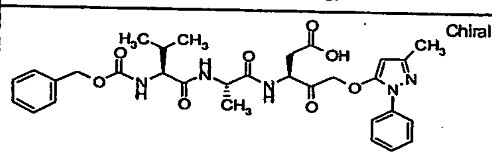
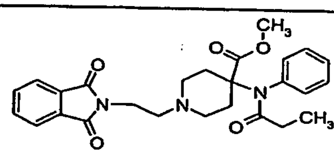
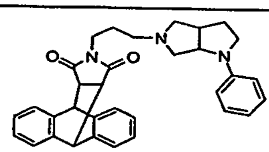
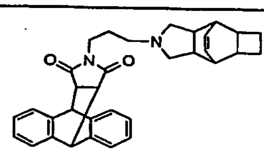
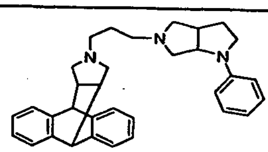
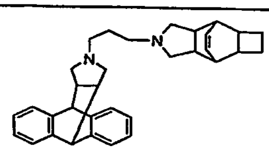
552		Greenwich Pharm.		WO 9411381
553		British Technol.		GB 2278842
554		Cell Therapeutics		WO 9416704
555		Cell Therapeutics		WO 9416704
556		Cell Therapeutics		WO 9416704
557		Cell Therapeutics		WO 9416704
558		Cell Therapeutics		WO 9416704
559		Cell Therapeutics		WO 9416704
560		Cell Therapeutics		WO 9416704
561		Otsuka		JP 94100561
562		Otsuka		JP 94100561

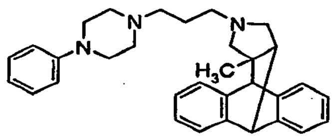
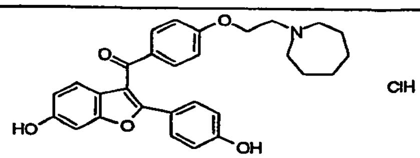
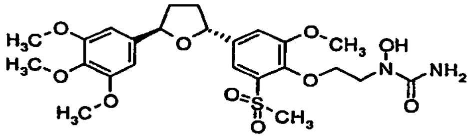
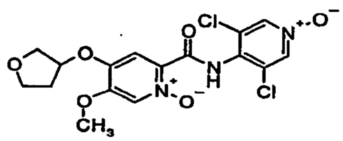
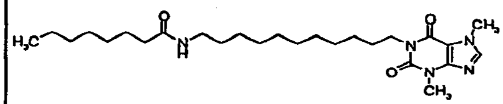
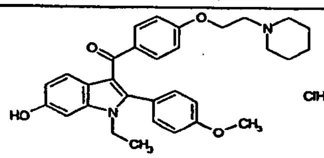
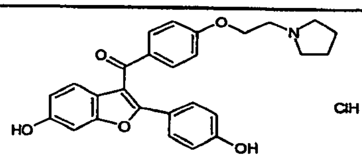
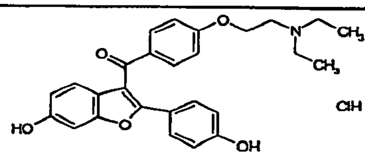
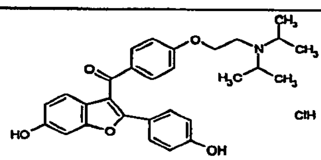
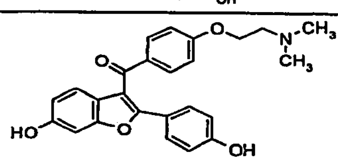
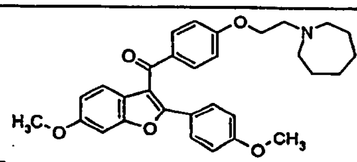
563		Otsuka		JP 94100561
564		Otsuka		JP 94100561
565		Otsuka		JP 94100561
566		Otsuka		JP 94100561
567		Otsuka		JP 94100561
568		Otsuka		JP 94100561
569		Otsuka		JP 94100561
570		Otsuka		JP 94100561
571		Abbott GmbH		WO 9500493
572		Abbott GmbH		WO 9500507
573		Leo		WO 9502577

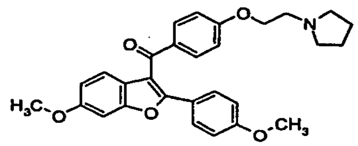
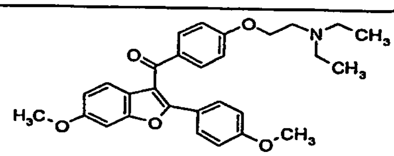
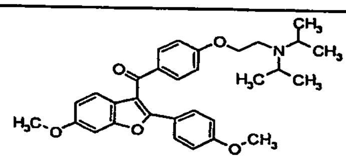
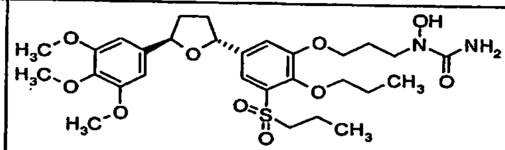
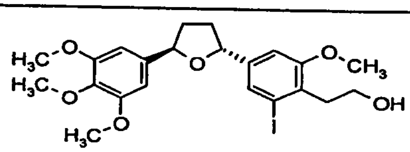
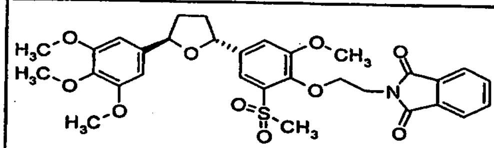
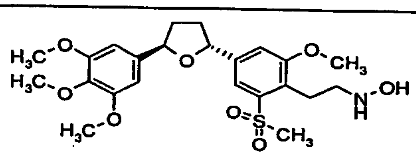
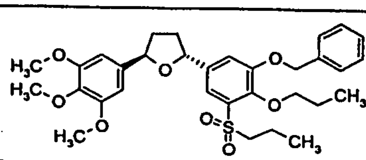
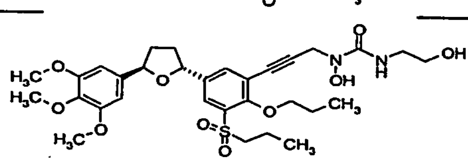
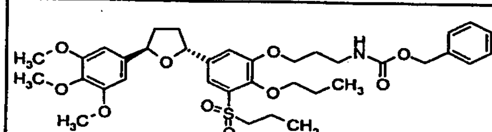
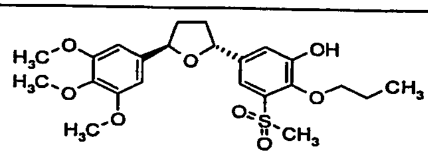
574		GlaxoSmithKline		WO 9504734
575		Kyorin	Kono, Y. et al. 115th Annu Meet Pharmaceut Soc Jpn (March 29-31, Sendai) 1995, Abst 30 (A3) 10-3 .	EP 538477
576		Abbott GmbH		WO 9500507
577		Abbott GmbH		WO 9500507
578		Abbott GmbH		WO 9500507
579		Abbott GmbH		WO 9500493
580		Abbott GmbH		WO 9500493
581		Abbott GmbH		WO 9500493
582		Abbott GmbH		WO 9500493
583		Abbott GmbH		WO 9500493
584		Abbott GmbH		WO 9500493

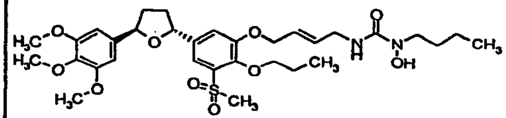
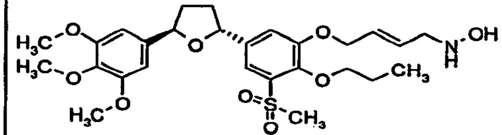
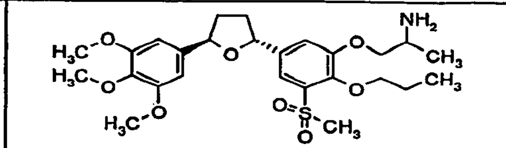
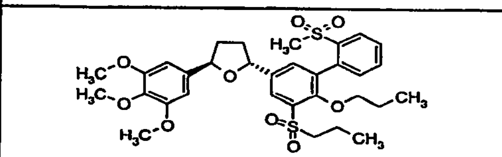
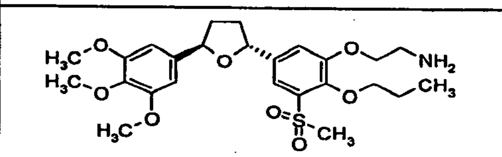
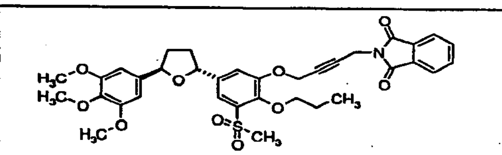
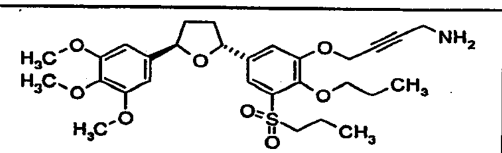
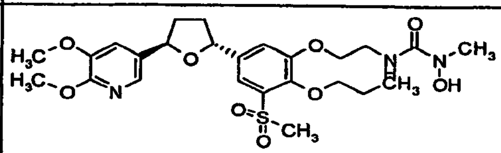
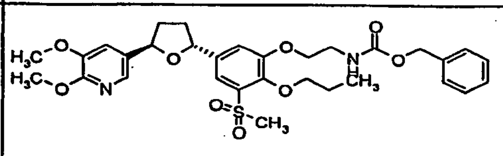
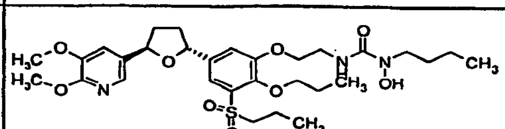
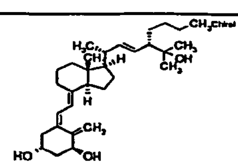
585		Abbott GmbH		WO 9500493
586		Abbott GmbH		WO 9500493
587		Abbott GmbH		WO 9500493
588		Abbott GmbH		WO 9500493
589		Abbott GmbH		WO 9500493
590		Sanofi-Synthelabo		EP 644197
591		Sanofi-Synthelabo		CA 2125021
592		Kyowa Hakko		WO 9509153
593		GlaxoSmithKline		WO 9504734
594		GlaxoSmithKline		WO 9504734
595		GlaxoSmithKline		WO 9504734

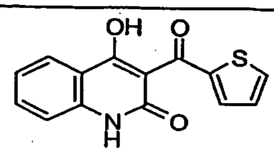
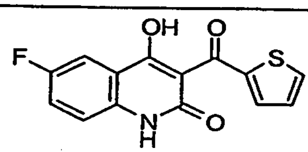
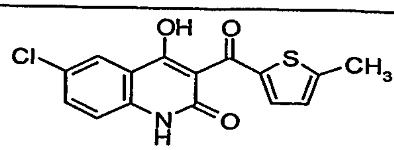
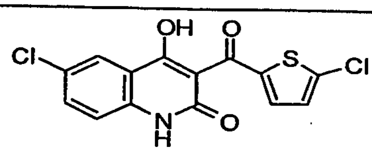
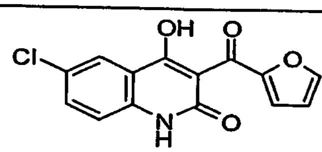
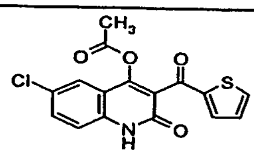
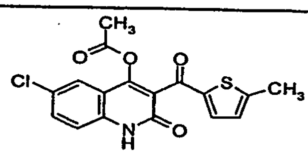
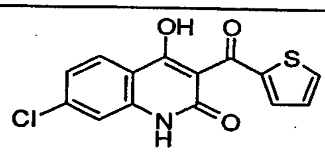
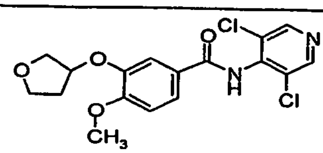
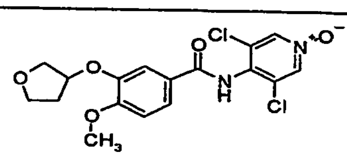
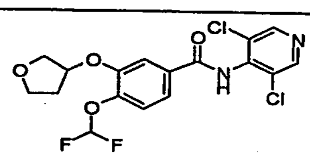
596		GlaxoSmithKline		WO 9504734
597		GlaxoSmithKline		WO 9504734
598		GlaxoSmithKline		WO 9504734
599		Japan Tobacco		JP 95002779
600		Pharmacia	1) Gozzi, P. et al. J Pharmacol Exp Ther 1999, 291(1): 199.	JP 1995501330
601		Bayer		US 5409932
602		Bayer		US 5411960
603		Sanofi-Synthelabo		EP 644197
604		Sanofi-Synthelabo		EP 644197
605		Sanofi-Synthelabo		EP 644197
606		Sanofi-Synthelabo		CA 2125021

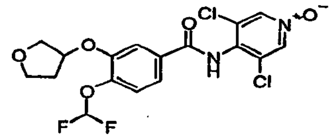
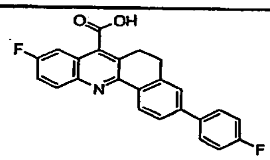
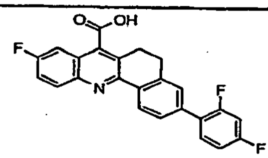
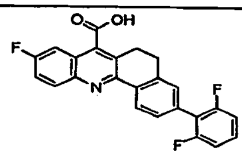
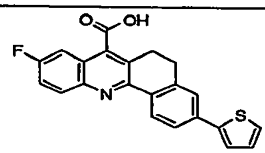
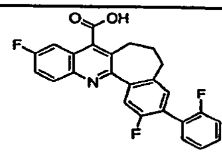
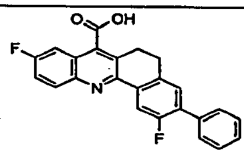
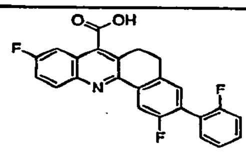
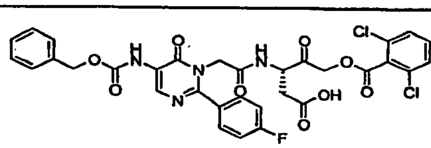
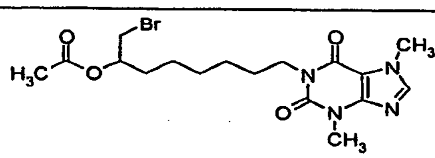
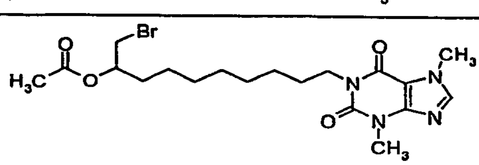
607		Sanofi-Synthelabo		CA 2125021
608		Sanofi-Synthelabo		CA 2125021
609		Sanofi-Synthelabo		CA 2125021
610		Sanofi-Synthelabo		CA 2125021
611		Sanofi-Synthelabo		CA 2125021
612		Sanofi-Synthelabo		CA 2125021
613		Baxter	France, C.P. et al. Drug Develop Res 1995, 35: 49.	EP 396282
614		Bayer		US 5411960
615		Bayer		US 5411960
616		Bayer		US 5411960
617		Bayer		US 5411960

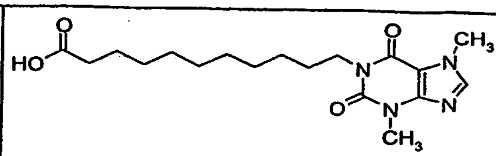
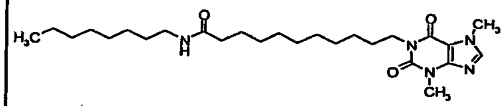
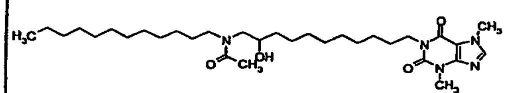
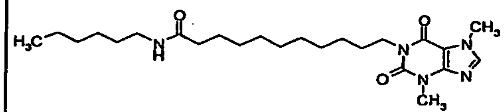
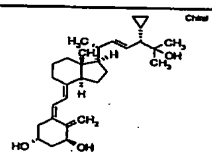
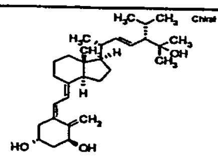
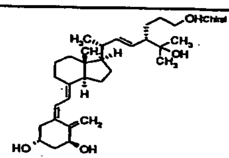
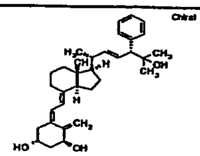
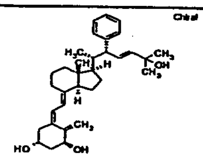
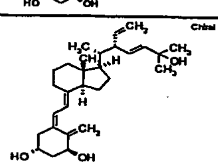
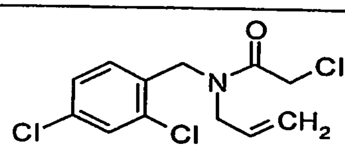
618		Bayer		US 5409932
619		Lilly		WO 9517382
620		Millennium		WO 9518610
621		Aventis Pharma		WO 9520578
622		Cell Therapeutics		WO 9522546
623		Lilly		WO 9517382
624		Lilly		WO 9517382
625		Lilly		WO 9517382
626		Lilly		WO 9517382
627		Lilly		WO 9517382
628		Lilly		WO 9517382

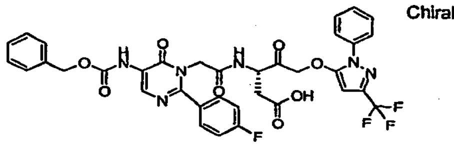
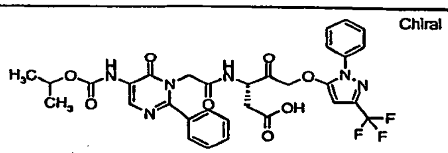
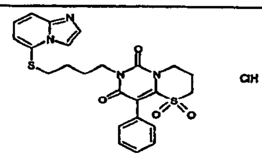
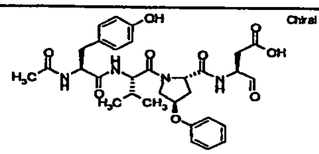
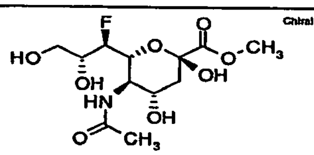
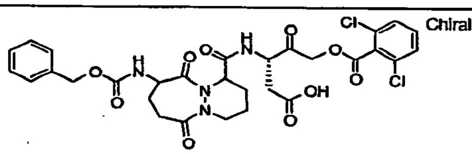
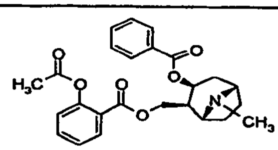
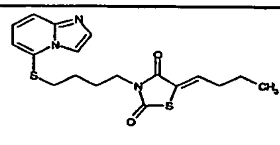
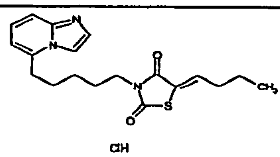
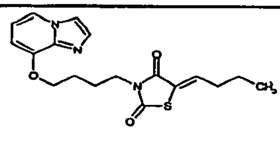
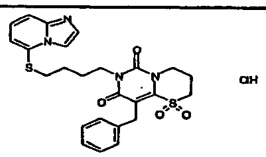
629		Lilly		WO 9517382
630		Lilly		WO 9517382
631		Lilly		WO 9517382
632		CytoMed		WO 9518610
633		CytoMed		WO 9518610
634		CytoMed		WO 9518610
635		CytoMed		WO 9518610
636		CytoMed		WO 9518610
637		CytoMed		WO 9518610
638		CytoMed		WO 9518610
639		CytoMed		WO 9518610

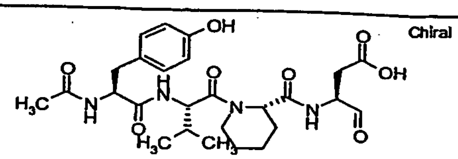
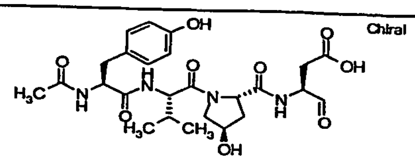
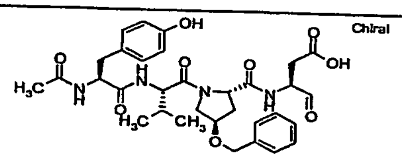
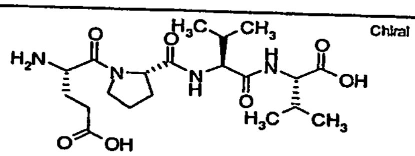
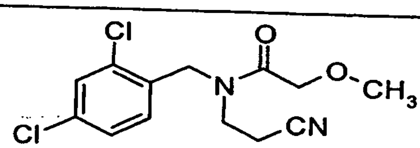
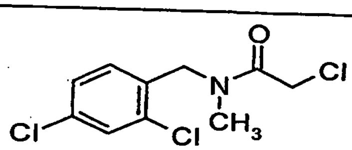
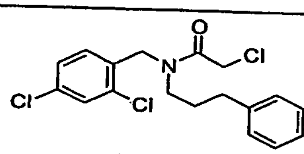
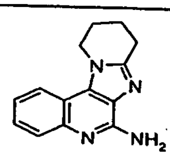
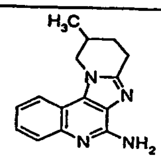
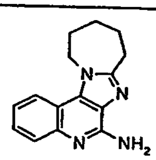
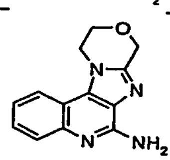
640		CytoMed		WO 9518610
641		CytoMed		WO 9518610
642		CytoMed		WO 9518610
643		CytoMed		WO 9518610
644		CytoMed		WO 9518610
645		CytoMed		WO 9518610
646		CytoMed		WO 9518610
647		CytoMed		WO 9518610
648		CytoMed		WO 9518610
649		CytoMed		WO 9518610
650		Duphar		EP 664287

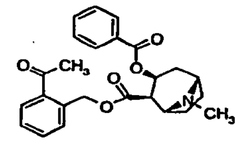
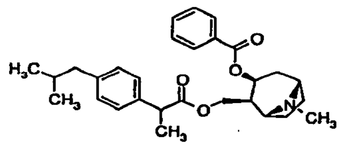
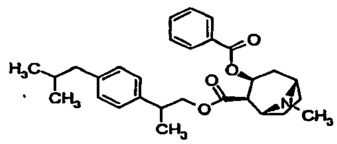
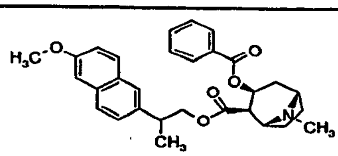
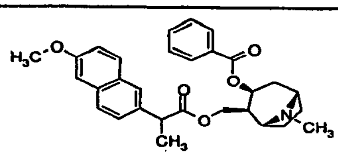
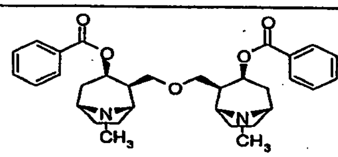
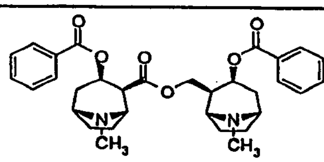
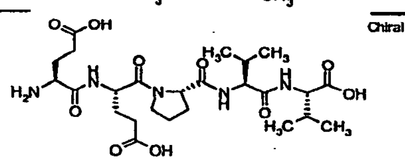
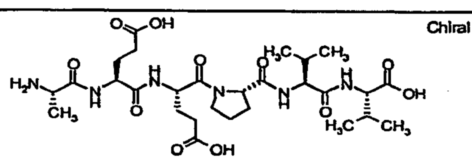
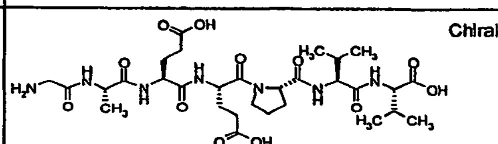
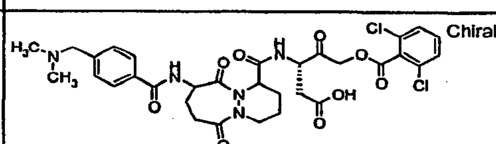
651		Japan Tobacco		JP 95002779
652		Japan Tobacco		JP 95002779
653		Japan Tobacco		JP 95002779
654		Japan Tobacco		JP 95002779
655		Japan Tobacco		JP 95002779
656		Japan Tobacco		JP 95002779
657		Japan Tobacco		JP 95002779
658		Japan Tobacco		JP 95002779
659		Aventis Pharma		WO 9520578
660		Aventis Pharma		WO 9520578
661		Aventis Pharma		WO 9520578

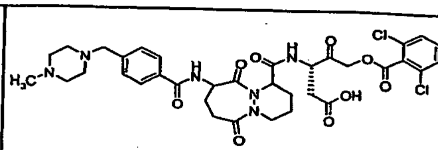
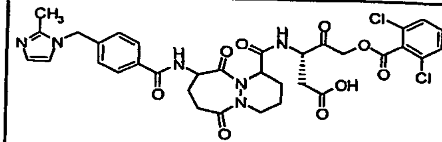
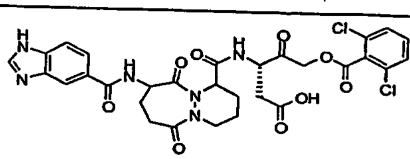
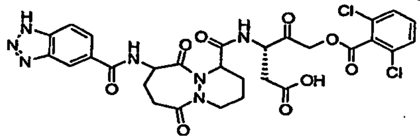
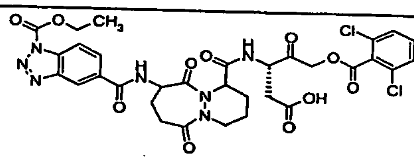
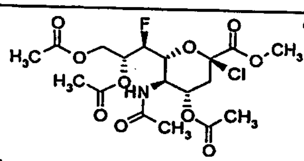
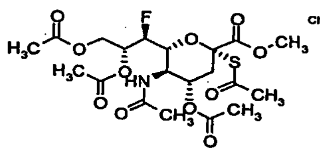
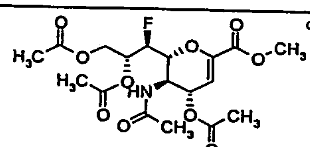
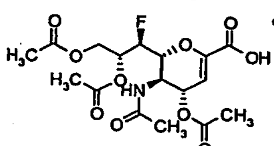
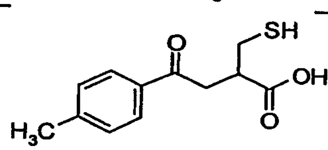
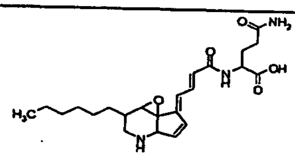
662		Aventis Pharma		WO 9520578
663		Kyowa Hakko		WO 9509153
664		Kyowa Hakko		WO 9509153
665		Kyowa Hakko		WO 9509153
666		Kyowa Hakko		WO 9509153
667		Kyowa Hakko		WO 9509153
668		Kyowa Hakko		WO 9509153
669		Kyowa Hakko		WO 9509153
670	 Chiral	Sanofi-Synthelabo		WO 9526958
671		Cell Therapeutics		WO 9522546
672		Cell Therapeutics		WO 9522546

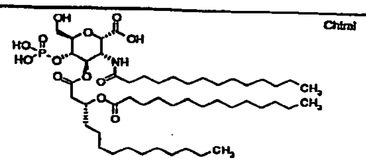
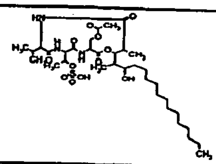
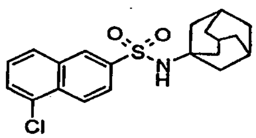
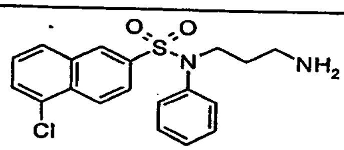
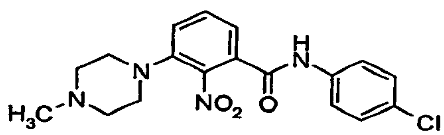
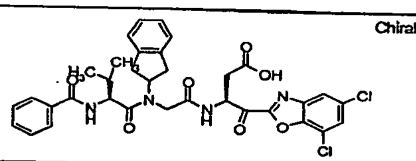
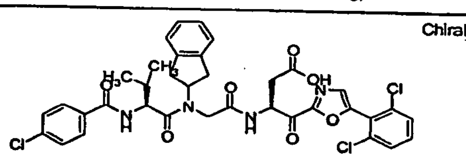
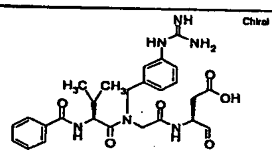
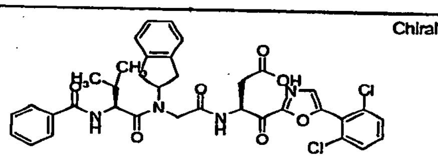
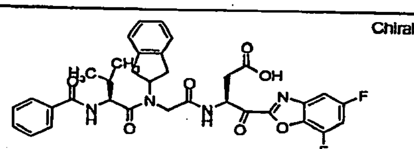
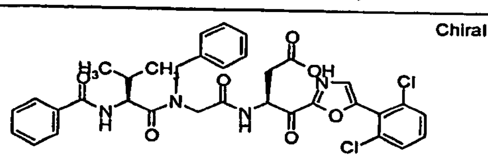
673		Cell Therapeutics		WO 9522546
674		Cell Therapeutics		WO 9522546
675		Cell Therapeutics		WO 9522546
676		Cell Therapeutics		WO 9522546
677		Duphar		EP 664287
678		Duphar		EP 664287
679		Duphar		EP 664287
680		Duphar		EP 664287
681		Duphar		EP 664287
682		Duphar		EP 664287
683		Sanofi-Synthelabo		WO 9529672

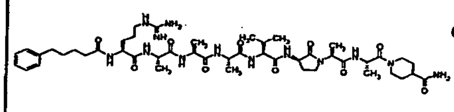
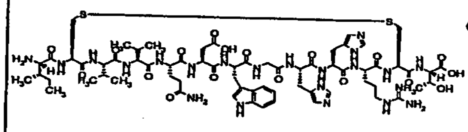
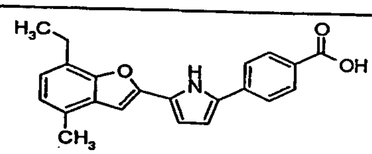
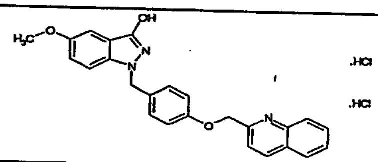
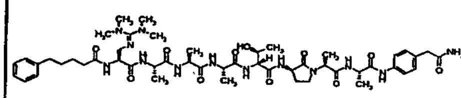
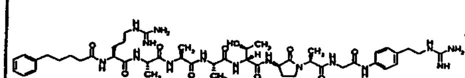
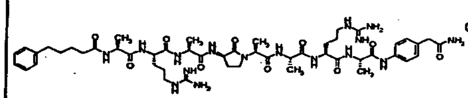
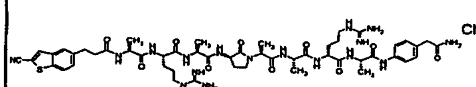
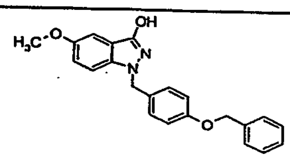
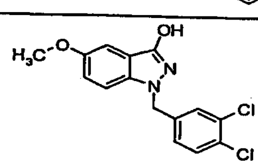
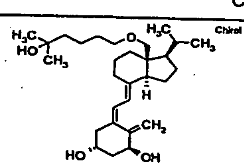
684		Sanofi-Synthelabo		WO 9526958
685		Sanofi-Synthelabo		WO 9526958
686		Takeda		WO 9535296
687		Vertex		WO 9535308
688		Daikin		EP 711766
689		Sanofi-Synthelabo		WO 9533751
690		Entropin		WO 9534561
691		Takeda		WO 9535296
692		Takeda		WO 9535296
693		Takeda		WO 9535296
694		Takeda		WO 9535296

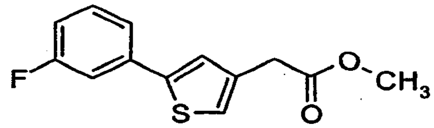
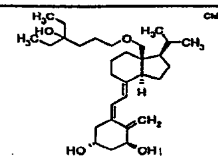
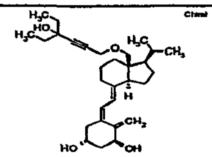
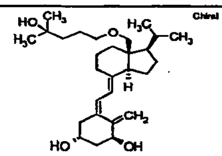
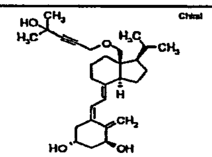
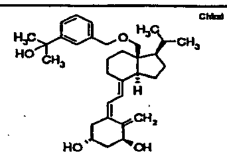
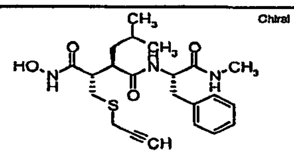
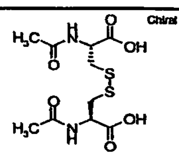
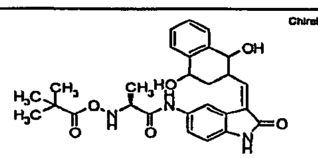
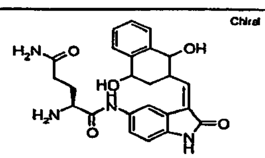
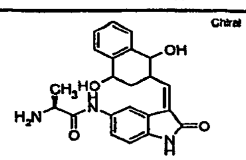
695		Vertex		WO 9535308
696		Vertex		WO 9535308
697		Vertex		WO 9535308
698		Ajinomoto		US 5464819
699		Sanofi-Synthelabo		WO 9529672
700		Sanofi-Synthelabo		WO 9529672
701		Sanofi-Synthelabo		WO 9529672
702		3M Pharmaceuticals		US 5482936
703		3M Pharmaceuticals		US 5482936
704		3M Pharmaceuticals		US 5482936
705		3M Pharmaceuticals		US 5482936

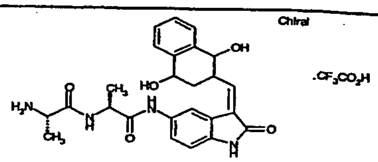
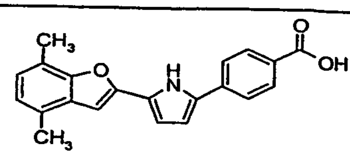
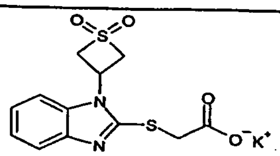
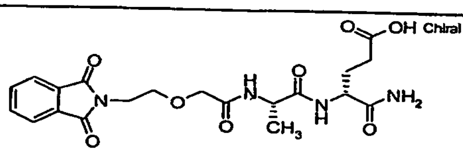
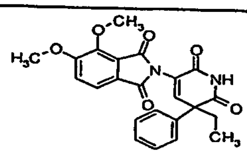
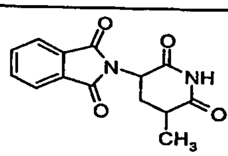
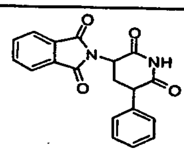
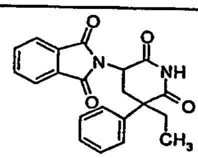
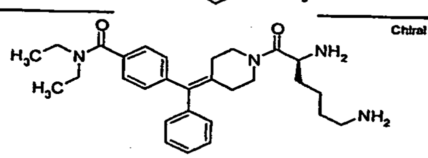
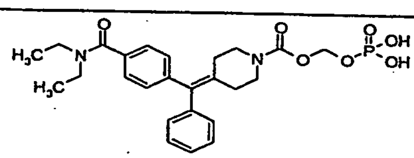
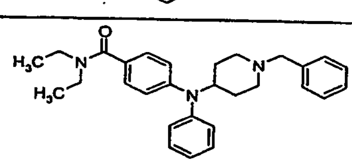
706		Entropin		WO 9534561
707		Entropin		WO 9534561
708		Entropin		WO 9534561
709		Entropin		WO 9534561
710		Entropin		WO 9534561
711		Entropin		WO 9534561
712		Entropin		WO 9534561
713		Ajinomoto		US 5464819
714		Ajinomoto		US 5464819
715		Ajinomoto		US 5464819
716		Sanofi-Synthelabo		WO 9533751

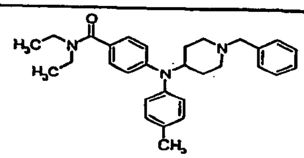
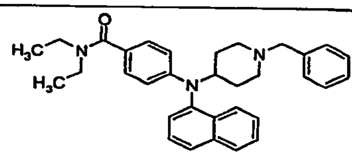
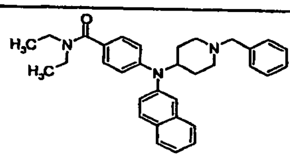
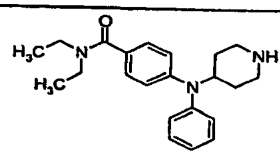
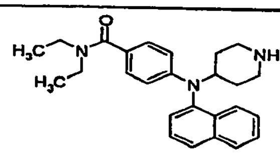
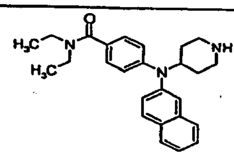
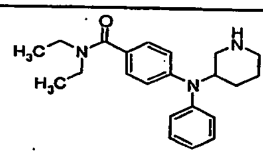
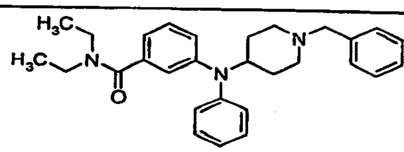
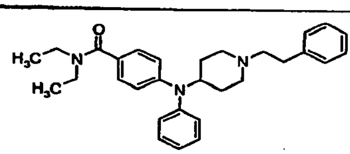
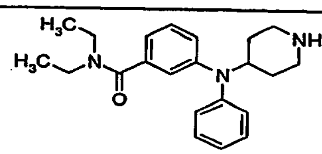
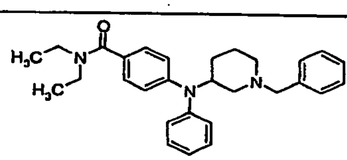
717	 Chiral	Sanofi-Synthelabo		WO 9533751
718	 Chiral	Sanofi-Synthelabo		WO 9533751
719	 Chiral	Sanofi-Synthelabo		WO 9533751
720	 Chiral	Sanofi-Synthelabo		WO 9533751
721	 Chiral	Sanofi-Synthelabo		WO 9533751
722	 Chiral	Daikin		EP 711766
723	 Chiral	Daikin		EP 711766
724	 Chiral	Daikin		EP 711766
725	 Chiral	Daikin		EP 711766
726		Taisho	1) Yoshida, H. et al. Biol Pharm Bull 1997, 20(1): 94.	JP 93051358
727		Microbial Chemistry Research Foundation		JP 96176157

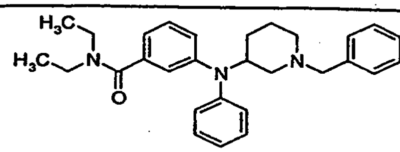
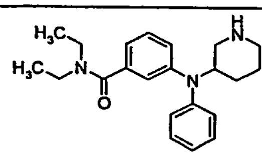
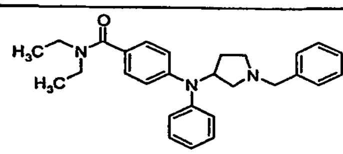
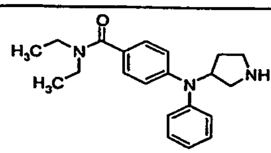
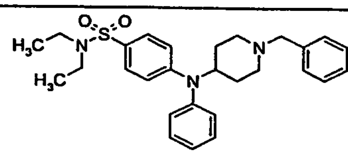
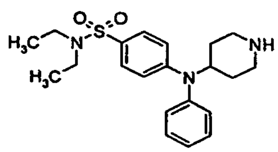
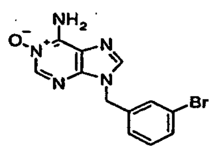
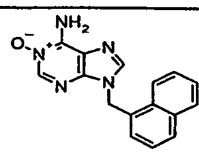
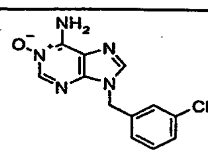
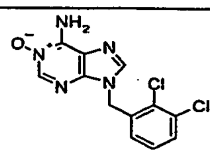
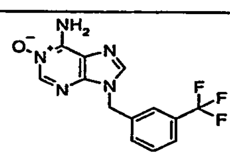
728		Sankyo	Shiozaki, M. et al. Tetrahedron Lett 1996 37(40): 7271.	
729		Nippon Kayaku		JP 96283290
730		Tanabe		WO 9640641
731		Tanabe		WO 9640641
732		Daiichi Pharmaceutical	1) Kawagoe, K. et al. AFMC Int Med Chem Symp (Sept 3-8, Tokyo) 1995, Abst P13M183.	JP 97059236
733		Vertex		WO 9722618
734		Vertex		WO 9722618
735		Vertex		WO 9722618
736		Vertex		WO 9722618
737		Vertex		WO 9722618
738		Vertex		WO 9722618

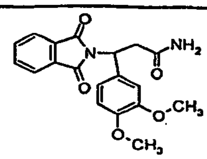
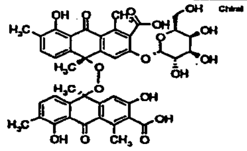
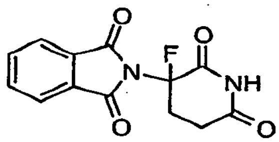
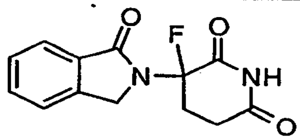
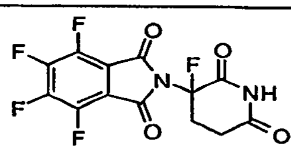
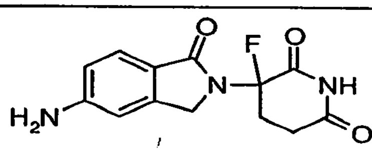
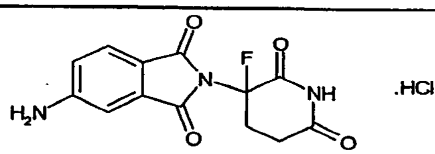
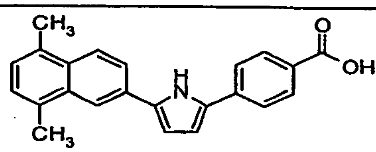
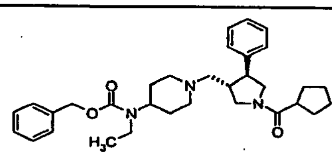
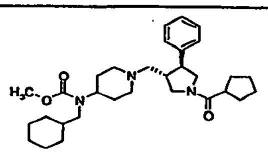
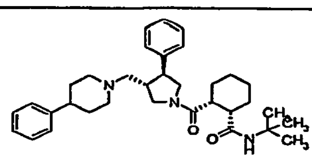
739	 Chiral	AstraZeneca		WO 9731023
740	 Chiral	University of Pennsylvania		WO 9733603
741		Eisai		EP 889032
742		AWD.pharma		WO 9734874
743	 Chiral	AstraZeneca		WO 9731023
744	 Chiral	AstraZeneca		WO 9731023
745	 Chiral	AstraZeneca		WO 9731023
746	 Chiral	AstraZeneca		WO 9731023
747		AWD.pharma		WO 9734874
748		AWD.pharma		WO 9734874
749		Leo		WO 9737972

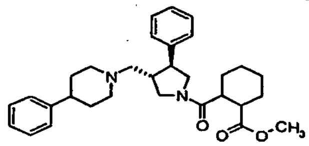
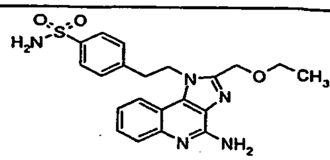
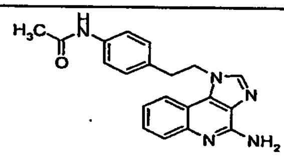
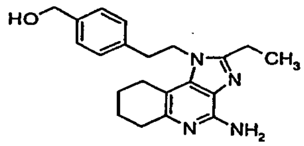
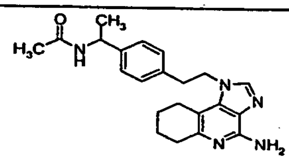
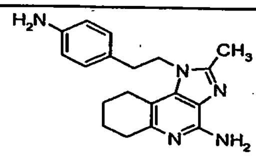
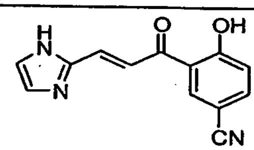
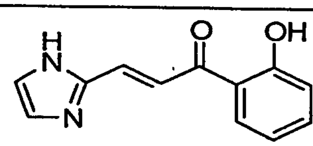
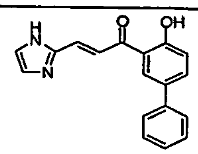
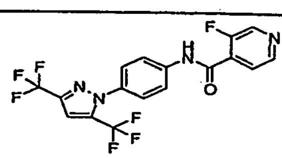
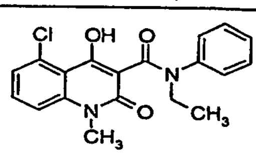
750		Taisho		JP 97194476
751		Leo		WO 9737972
752		Leo		WO 9737972
753		Leo		WO 9737972
754		Leo		WO 9737972
755		Leo		WO 9737972
756		GlaxoSmithKline		WO 9743250
757		AstraZeneca	1) Saemstrand, B. et al. J Pharmacol Exp Ther 1999, 288(3): 1174.	EP 0463514
758		Pharmacia		WO 9745409
759		Pharmacia		WO 9745409
760		Pharmacia		WO 9745409

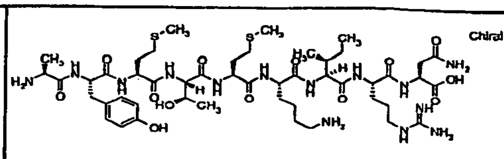
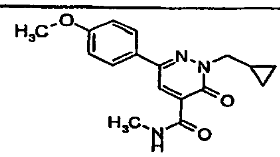
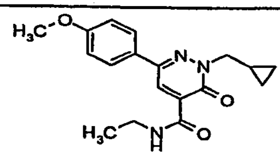
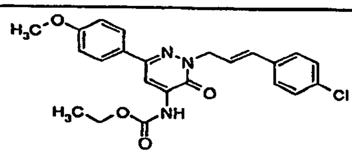
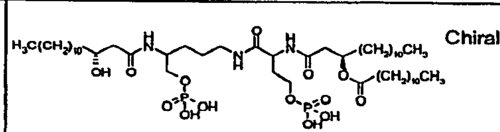
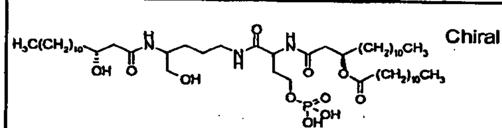
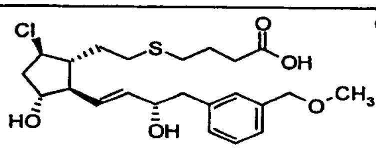
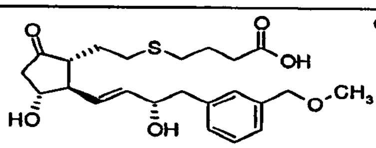
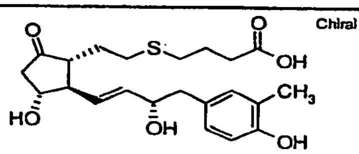
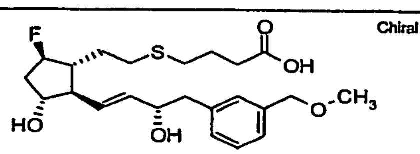
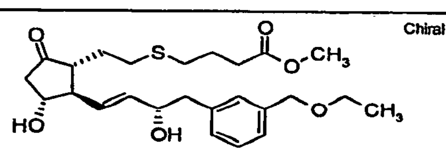
761		Pharmacia		WO 9745409
762		Eisai	Nagai, M. et al. 217th ACS Natl Meet (March 21-25, Anaheim) 1999, Abst MEDI 050.	EP 889032
763		Bashkir Medical University	Sadykov, R.F. et al. Naunyn-Schmied Arch Pharmacol 1998, 358(1, Suppl. 2): Abst P 52.28.	
764		LEK		EP 477912
765		Gruenenthal		EP 856513
766		Gruenenthal		EP 856513
767		Gruenenthal		EP 856513
768		Gruenenthal		EP 856513
769		AstraZeneca		WO 9828275
770		AstraZeneca		WO 9828275
771		AstraZeneca		WO 9828270

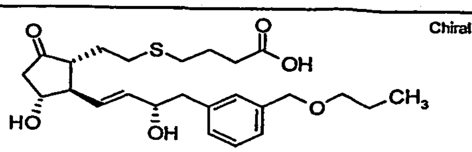
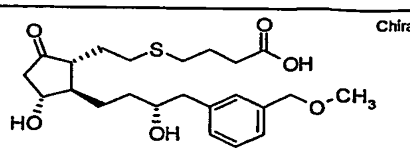
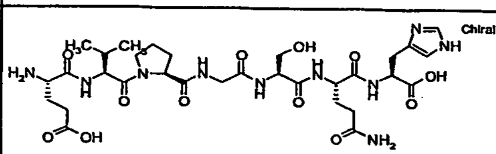
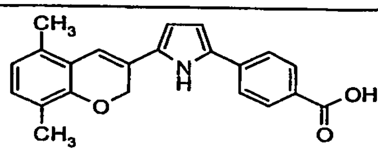
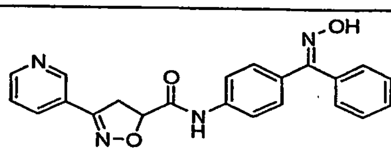
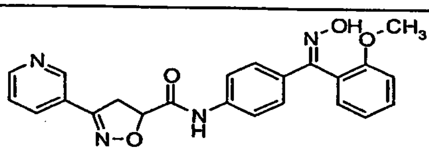
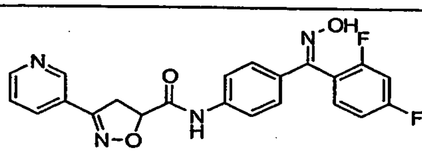
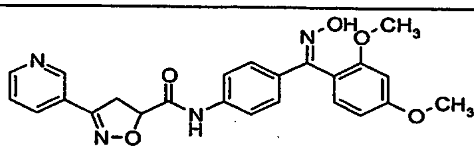
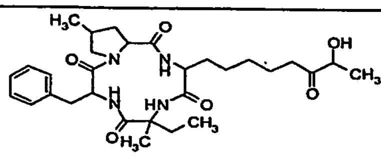
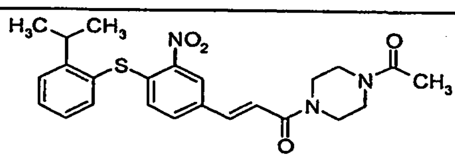
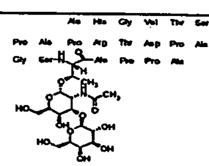
772		AstraZeneca		WO 9828270
773		AstraZeneca		WO 9828270
774		AstraZeneca		WO 9828270
775		AstraZeneca		WO 9828270
776		AstraZeneca		WO 9828270
777		AstraZeneca		WO 9828270
778		AstraZeneca		WO 9828270
779		AstraZeneca		WO 9828270
780		AstraZeneca		WO 9828270
781		AstraZeneca		WO 9828270
782		AstraZeneca		WO 9828270

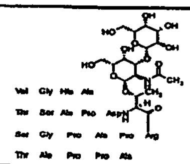
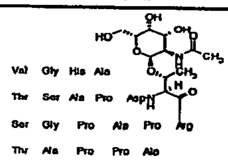
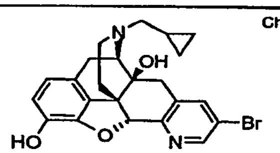
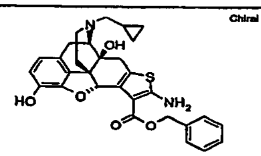
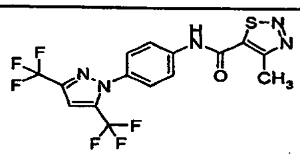
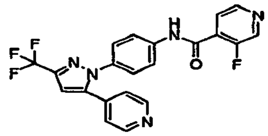
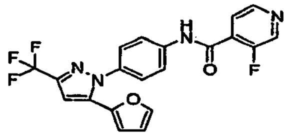
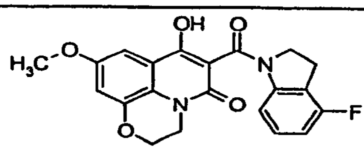
783		AstraZeneca		WO 9828270
784		AstraZeneca		WO 9828270
785		AstraZeneca		WO 9828270
786		AstraZeneca		WO 9828270
787		AstraZeneca		WO 9828270
788		AstraZeneca		WO 9828270
789		Japan Energy		JP 98231297
790		Japan Energy		JP 98231297
791		Japan Energy		JP 98231297
792		Japan Energy		JP 98231297
793		Japan Energy		JP 98231297

794		Celgene	1) Moreira, A.L. et al. J Neuro-Oncol 1999, 43(2): 109.	US 5463063
795		Daiichi Pharmaceutical	Koiwa, T. et al. J Antibiot 1999, 52(2): 198.	
796		Celgene		US 5874448
797		Celgene		US 5874448
798		Celgene		US 5874448
799		Celgene		US 5874448
800		Celgene		US 5874448
801		Eisai		EP 889032
802		Merck & Co.		WO 9909984
803		Merck & Co.		WO 9909984
804		Merck & Co.		WO 9909984

805		Merck & Co.		WO 9909984
806		Hokuriku		JP 99080156
807		Hokuriku		JP 99080156
808		Hokuriku		JP 99080156
809		Hokuriku		JP 99080156
810		Hokuriku		JP 99080156
811		SSP		CA 2255337
812		SSP		CA 2255337
813		SSP		CA 2255337
814		Abbott	Madar, D. et al. 222nd ACS Natl Meet (Aug 26-30, Chicago) 2001, Abst MEDI 7.	EP 1068187
815		Active Biotech		WO 9955678

816		Amersham	1) Gesser, B. et al. Proc Natl Acad Sci USA 1997, 94(26): 14620.	WO 9601318
817		Kowa		WO 9944995
818		Kowa		WO 9944995
819		Kowa		WO 9944995
820		OM Pharma		WO 0000462
821		OM Pharma		WO 0000462
822		Ono		WO 0003980
823		Ono		WO 0003980
824		Ono		WO 0003980
825		Ono		WO 0003980
826		Ono		WO 0003980

827		Ono		WO 0003980
828		Ono		WO 0003980
829		University of Bristol		WO 0014114
830		Eisai	Hibi, S. et al. Bioorg Med Chem Lett 2000, 10(7): 623.	EP 0889032
831		Janssen		WO 0021959
832		Janssen		WO 0021959
833		Janssen		WO 0021959
834		Janssen		WO 0021959
835		Fujisawa		WO 0021979
836		Abbott	1) Liu, G. et al. 220th ACS Natl Meet (Aug 20-24, Washington DC) 2000, Abst MEDI 171.	1) US 6110922
837		Cancer Research UK		WO 0052046

838		Cancer Research UK		WO 0052046
839		Cancer Research UK		WO 0052046
840		Southern Research Institute		WO 0112197
841		Southern Research Institute		WO 0112197
842		Abbott Labs.		EP 1068187
843		Abbott		EP 1068187
844		Abbott		EP 1068187
845		Fujisawa	Spears, G. et al. 21st Symp Med Chem (Nov 28-30, Kyoto) 2001, Abst 2P-29.	
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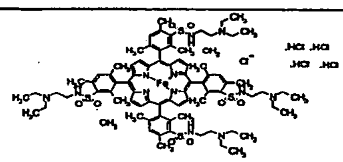
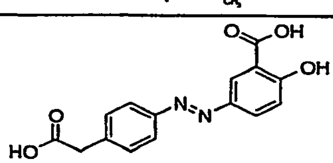
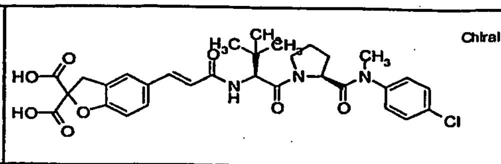
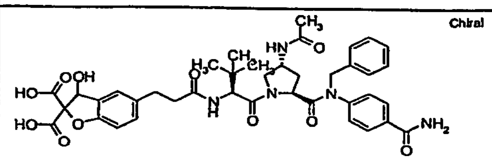
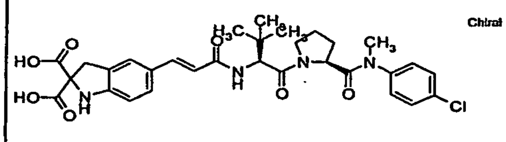
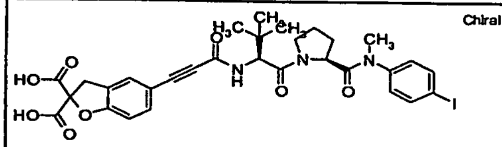
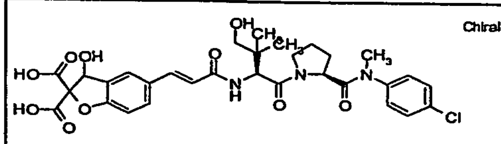
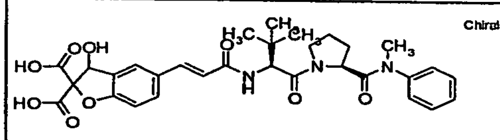
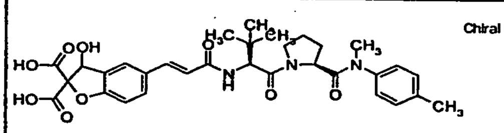
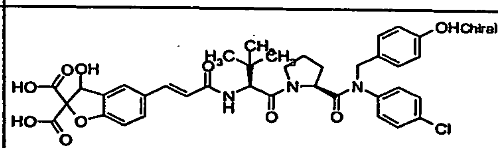
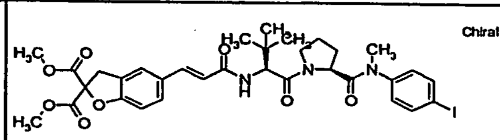
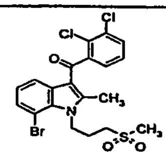
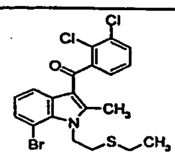
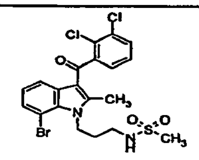
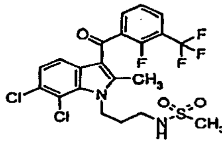
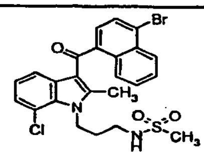
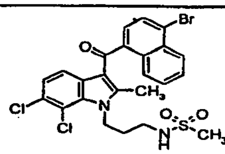
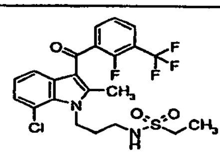
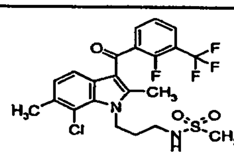
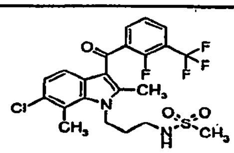
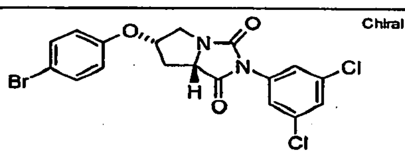
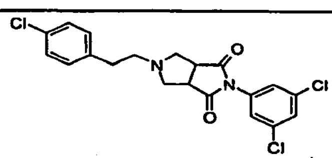
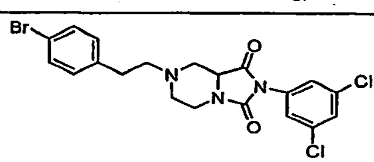
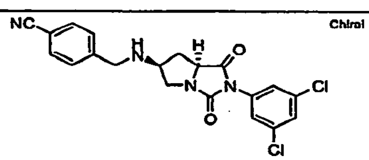
847		Eukarion		WO 0204454
848		Nobex	1) Riggs-Sauthier, J.A. et al. 224th ACS Natl Meet (Aug 18-22, Boston) 2002, Abst MEDI 305.	WO 0218324

849		Tularik		WO 0238107
850		Tularik		WO 0238107
851		Tularik		WO 0238107
852		Tularik		WO 0238107
853		Tularik		WO 0238107
854		Tularik		WO 0238107
855		Tularik		WO 0238107
856		Tularik		WO 0238107
857		Tularik		WO 0238107
858		Sanofi-Synthelabo		WO 0242269
859		Sanofi-Synthelabo		WO 0242269

860		Sanofi-Synthelabo		WO 0242269
861		Sanofi-Synthelabo		WO 0242269
862		Sanofi-Synthelabo		WO 0242269
863		Sanofi-Synthelabo		WO 0242269
864		Sanofi-Synthelabo		WO 0242269
865		Sanofi-Synthelabo		WO 0242269
866		Sanofi-Synthelabo		WO 0242269
867		Bristol-Myers Squibb		WO 0244181
868		Bristol-Myers Squibb		WO 0244181
869		Bristol-Myers Squibb		WO 0244181
870		Bristol-Myers Squibb		WO 0244181

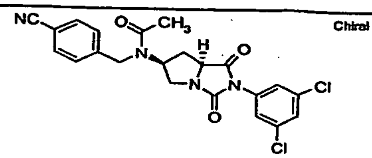
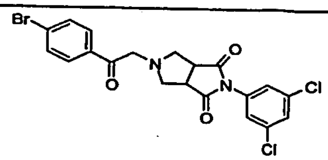
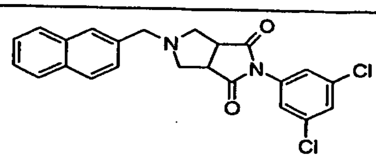
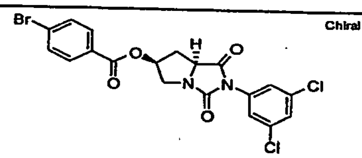
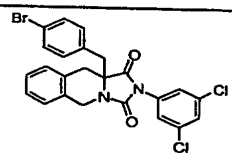
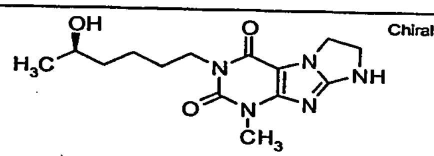
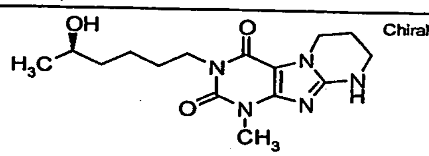
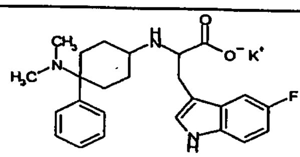
871		Bristol-Myers Squibb		WO 0244181
872		Bristol-Myers Squibb		WO 0244181
873		Bristol-Myers Squibb		WO 0244181
874		Bristol-Myers Squibb		WO 0244181
875		Bristol-Myers Squibb		WO 0244181
876		Cell Therapeutics		WO 0268421
877		Cell Therapeutics		WO 0268421
878		Gruenenthal		WO 0290317

Table 35 Continued

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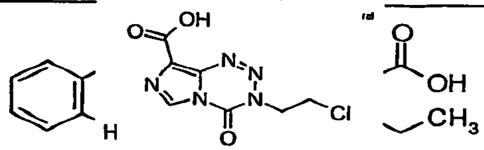
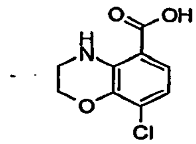
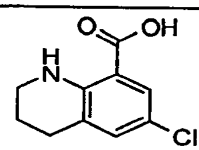
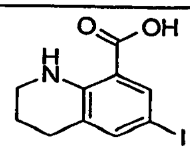
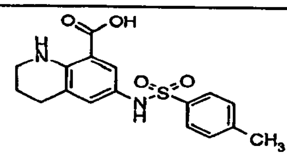
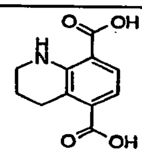
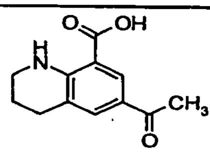
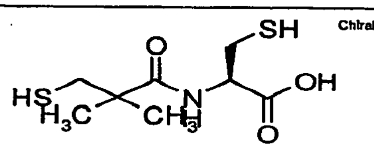
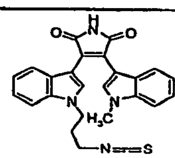
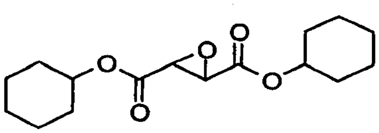
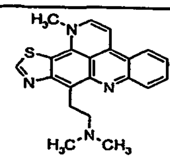
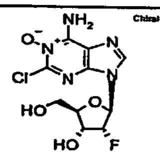
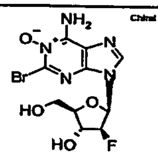
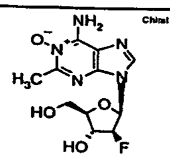
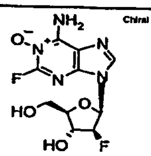
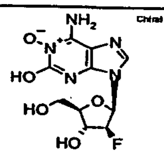
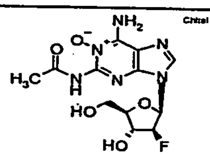
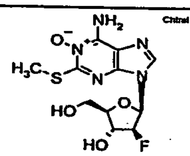
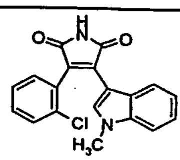
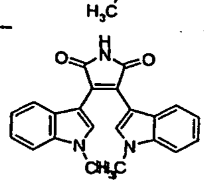
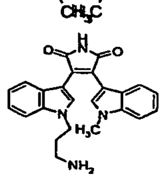
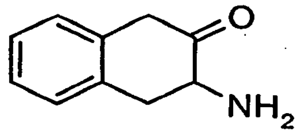
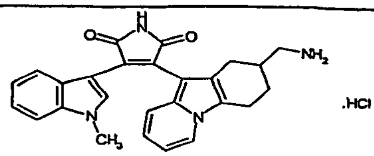
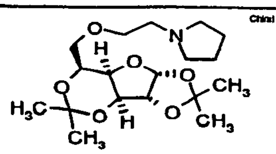
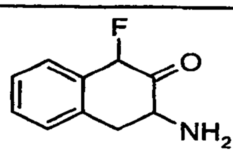
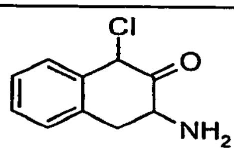
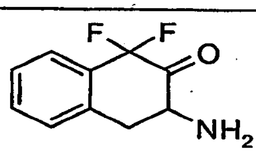
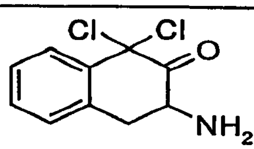
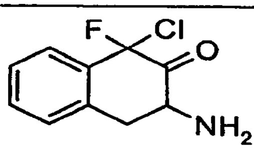
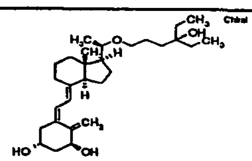
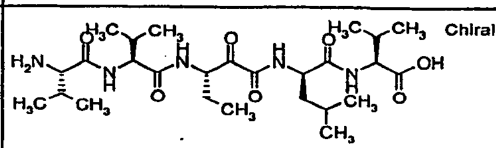
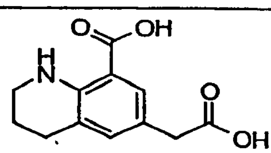
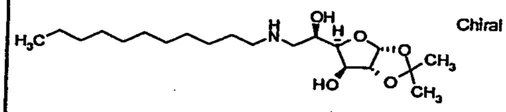
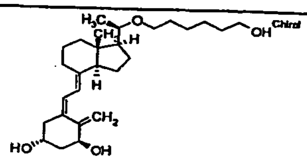
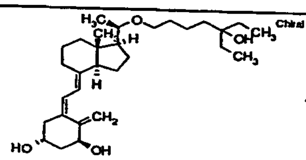
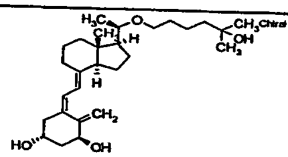
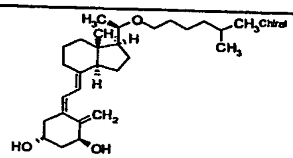
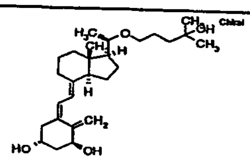
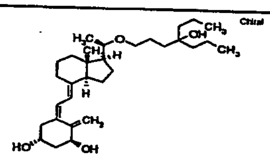
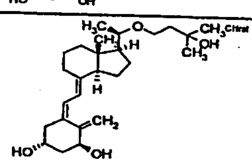
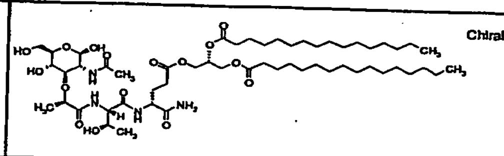
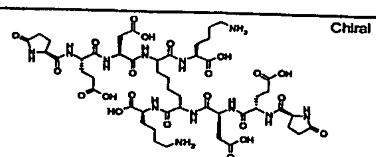
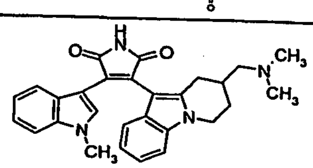
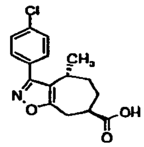
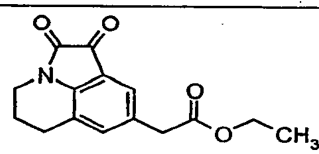
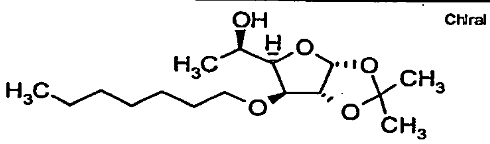
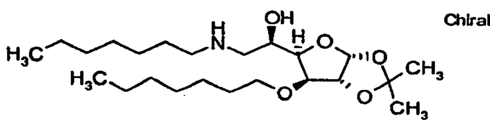
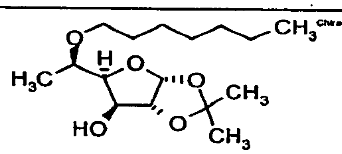
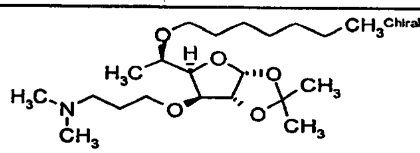
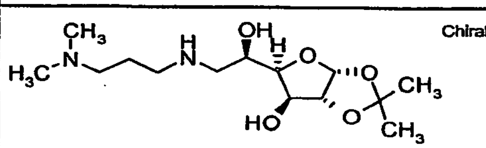
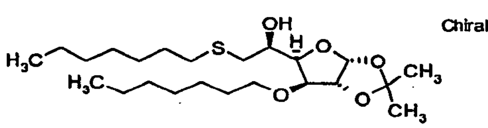
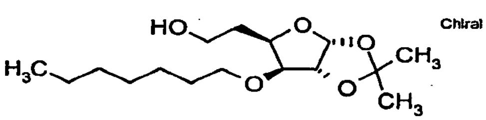
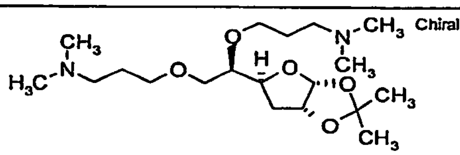
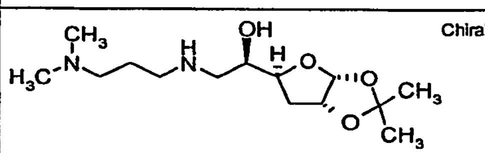
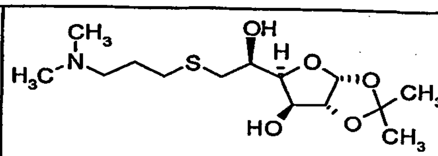
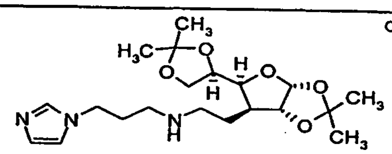
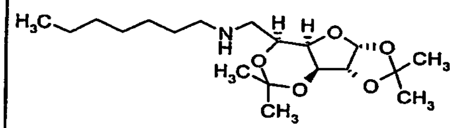
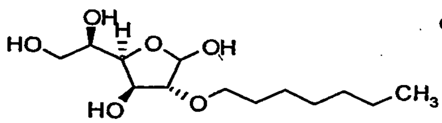
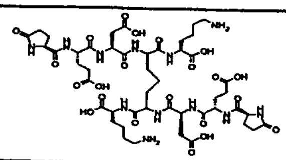
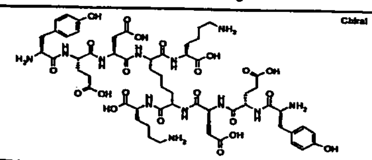
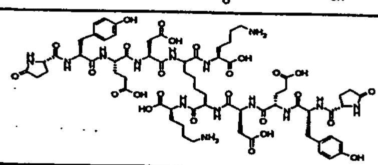
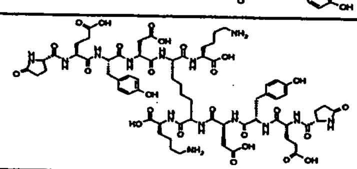
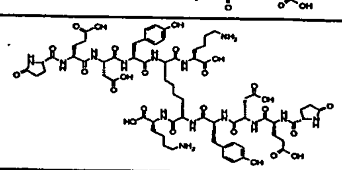
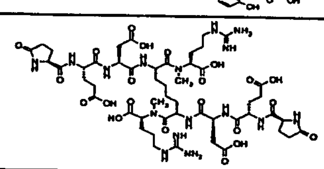
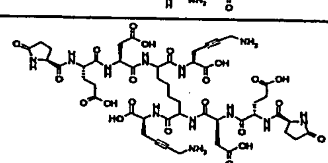
881		May & Baker		EP 252682
882		Kyorin		EP 310096
883		Kyorin		EP 310096
884		Kyorin		EP 310096
885		Kyorin		EP 310096
886		Kyorin		EP 310096
887		Kyorin		EP 310096
888		Santen		EP 326326
889		Roche		AU 8929658
890		Taisho	1) Takeshita, K. et al. Int J Immunother 1988, 4(2): 97-106.	JP 79141750

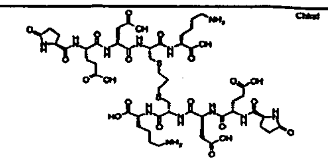
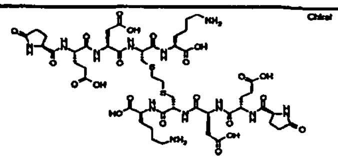
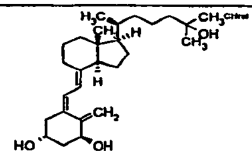
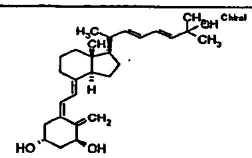
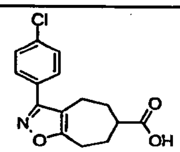
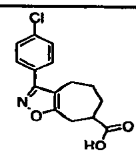
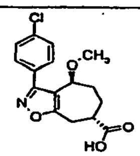
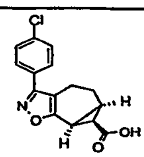
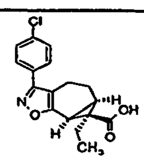
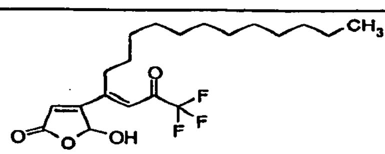
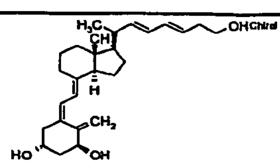
891		Harbor Branch Found.	1) Burres, N.S. et al. Proc Amer Assoc Cancer Res 1989, 30: Abst 1914.	EP 331320
892		Scripps Clinic Res. Found.		WO 8908658
893		Scripps Clinic Res. Found.		WO 8908658
894		Scripps Clinic Res. Found.		WO 8908658
895		Scripps Clinic Res. Found.		WO 8908658
896		Scripps Clinic Res. Found.		WO 8908658
897		Scripps Clinic Res. Found.		WO 8908658
898		Scripps Clinic Res. Found.		WO 8908658
899		Roche		AU 8929658
900		Roche		AU 8929658
901		Roche		AU 8929658

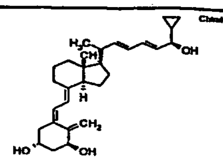
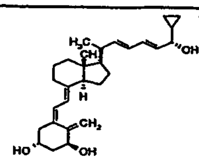
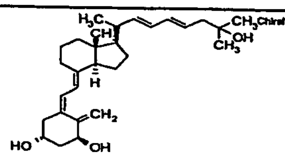
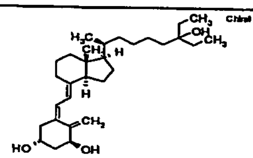
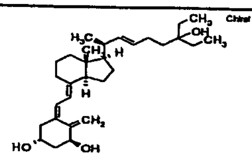
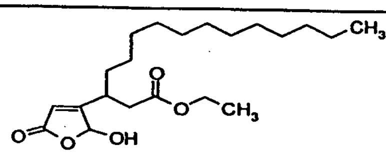
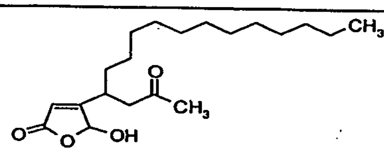
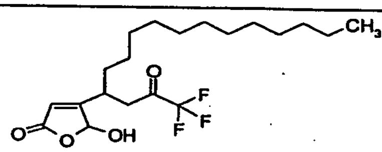
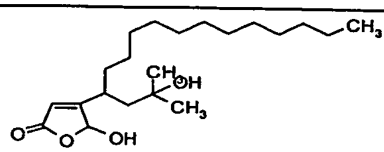
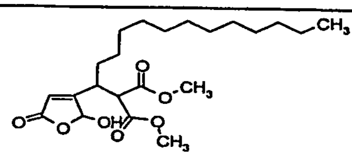
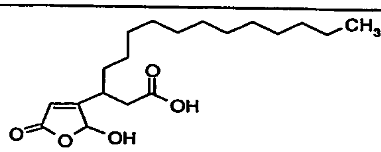
902		Aventis Pharma		EP 378456
903		Roche		EP 384349
904		Greenwich Pharm.		AU 9047648
905		Aventis Pharma		EP 378456
906		Aventis Pharma		EP 378456
907		Aventis Pharma		EP 378456
908		Aventis Pharma		EP 378456
909		Aventis Pharma		EP 378456
910		Leo	Binderup, L. et al. Biochem Pharmacol 1991, 42(8): 1569.	EP 460032
911		Microbial Chemistry Research Foundation	Muraoka, Y. et al. 30th Interscl Conf Antimicrob Agents Chemother (Oct 21-24, Atlanta) 1990, Abst 801 .	
912		Kyorin		AU 9057029

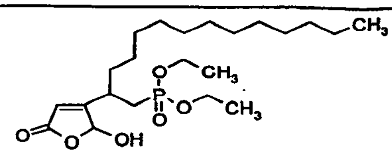
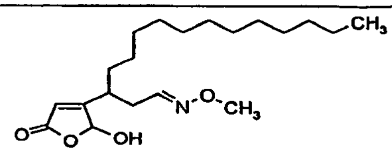
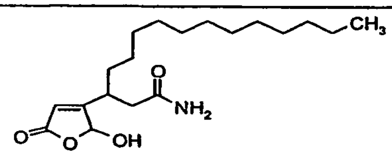
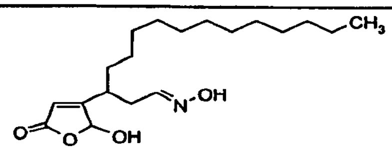
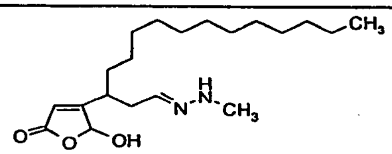
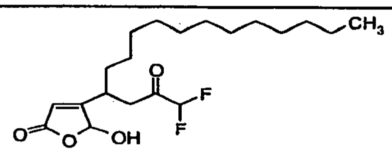
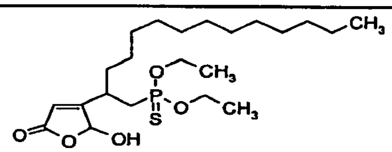
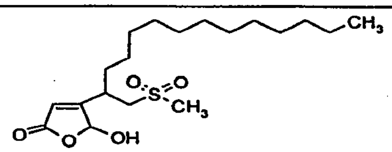
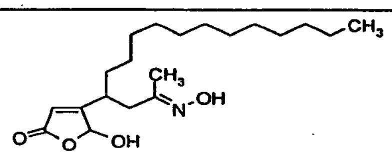
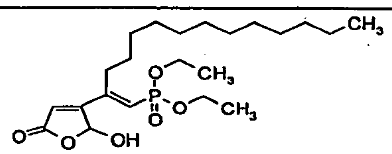
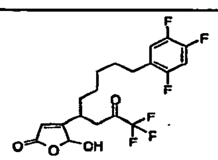
913		Greenwich Pharm.		AU 9057691
914		Leo		EP 460032
915		Leo		EP 460032
916		Leo		EP 460032
917		Leo		EP 460032
918		Leo		EP 460032
919		Leo		EP 460032
920		Leo		EP 460032
921		Novartis	Kricek, F. et al. Immunopharmacology 1997, 36(1): 27.	AU 9057875
922		Amersham Health	1) Frey, C.L. et al. 31st Intersci Conf Antimicrob Agents Chemother (Sept 29-Oct 2, Chicago) 1991, Abst 85 .	AU 9059014
923		Roche		EP 384349

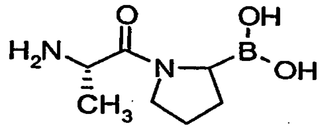
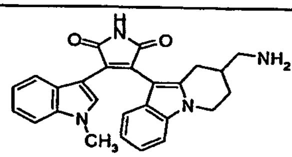
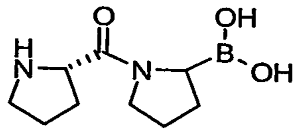
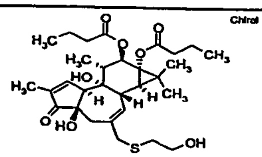
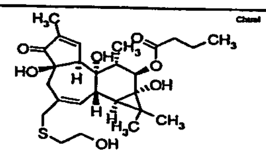
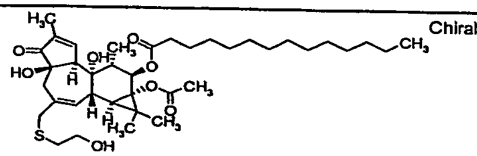
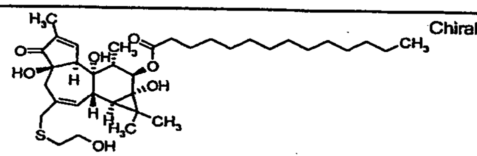
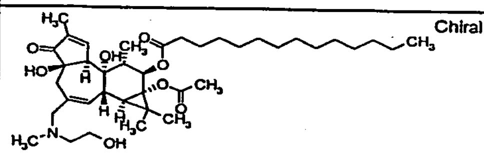
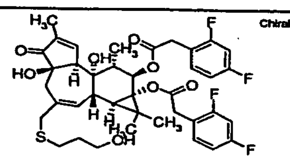
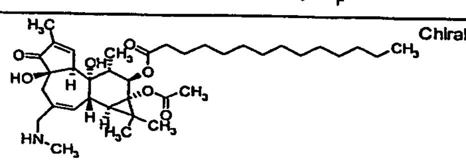
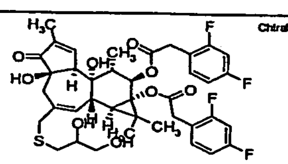
924		Roche		AU 9059154
925		Kyorin		AU 9057029
926		Greenwich Pharm.		AU 9057691
927		Greenwich Pharm.		AU 9057691
928		Greenwich Pharm.		AU 9057691
929		Greenwich Pharm.		AU 9057691
930		Greenwich Pharm.		AU 9057691
931		Greenwich Pharm.		AU 9057691
932		Greenwich Pharm.		AU 9057691
933		Greenwich Pharm.		AU 9057691
934		Greenwich Pharm.		AU 9057691

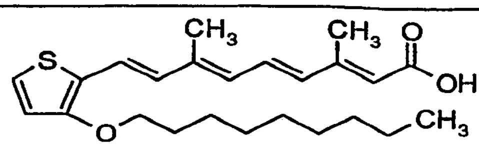
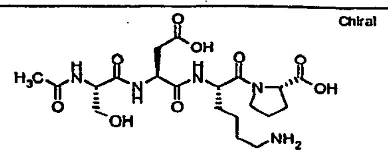
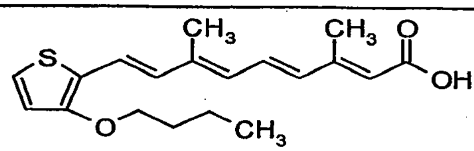
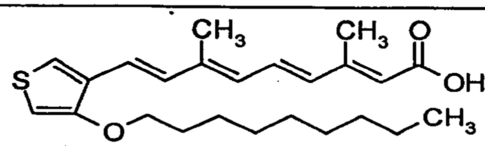
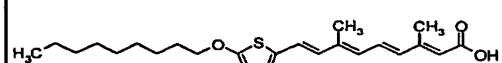
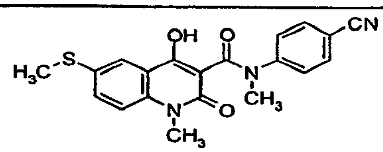
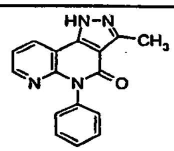
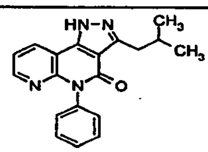
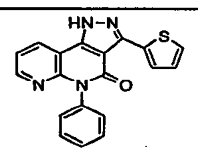
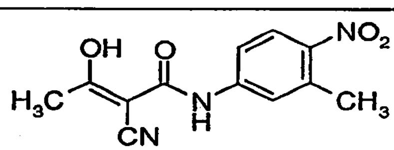
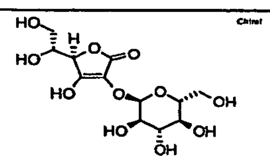
935	 Chiral	Greenwich Pharm.		AU 9057691
936	 Chiral	Greenwich Pharm.		AU 9057691
937	 Chiral	Greenwich Pharm.		AU 9057691
938	 Chiral	Greenwich Pharm.		AU 9057691
939		Amersham Health		AU 9059014
940	 Chiral	Amersham Health		AU 9059014
941		Amersham Health		AU 9059014
942		Amersham Health		AU 9059014
943		Amersham Health		AU 9059014
944		Amersham Health		AU 9059014
945		Amersham Health		AU 9059014

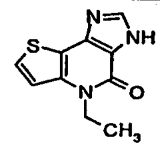
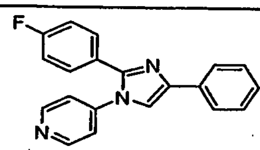
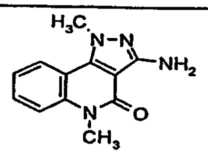
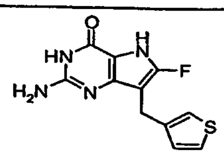
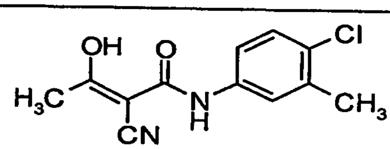
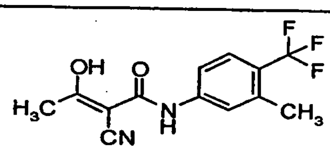
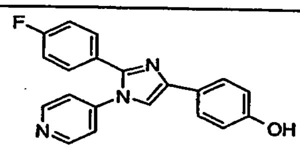
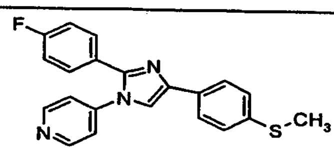
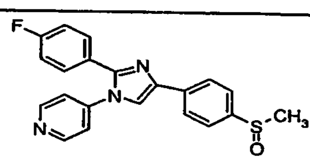
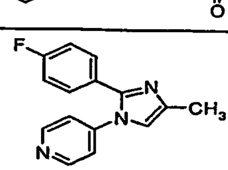
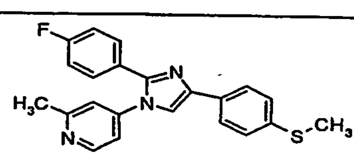
946		Amersham Health		AU 9059014
947		Amersham Health		AU 9059014
948		Leo	1) Elstner, E. et al. Blood 1994, 84(6): 1960.	1) EP 479871
949		Leo		WO 9100855
950		Roche		AU 9059154
951		Roche		AU 9059154
952		Roche		AU 9059154
953		Roche		AU 9059154
954		Roche		AU 9059154
955		Allergan		US 5013850
956		Leo		WO 9100855

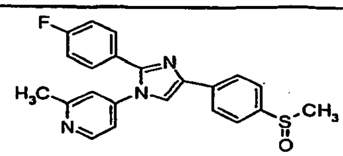
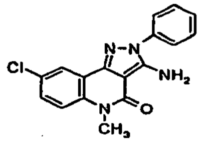
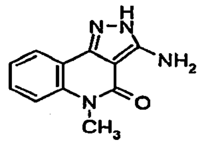
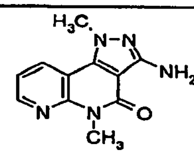
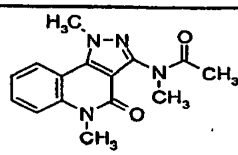
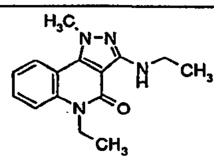
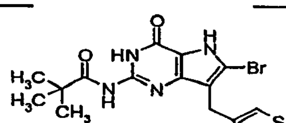
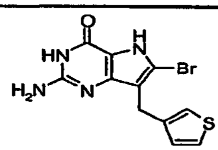
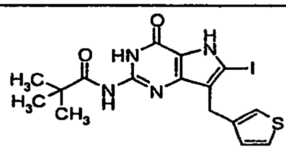
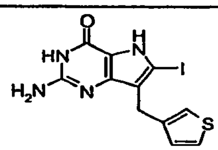
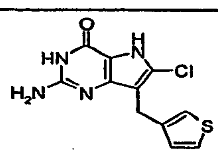
957		Leo		WO 9100855
958		Leo		WO 9100855
959		Leo		WO 9100855
960		Leo		EP 479871
961		Leo		EP 479871
962		Allergan		US 5013850
963		Allergan		US 5013850
964		Allergan		US 5013850
965		Allergan		US 5013850
966		Allergan		US 5013850
967		Allergan		US 5013850

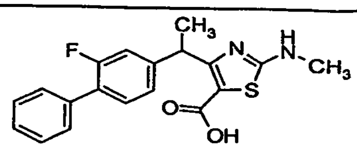
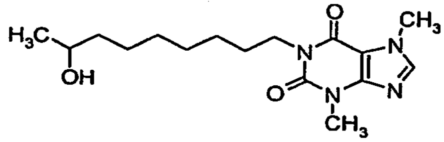
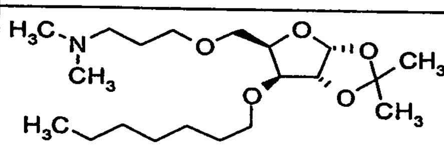
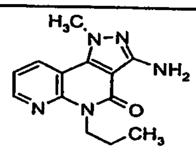
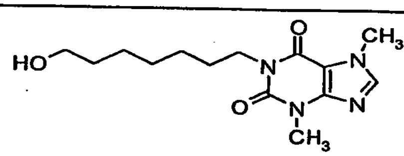
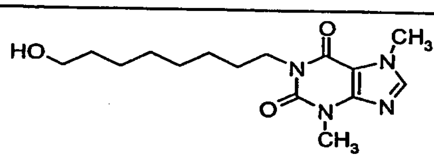
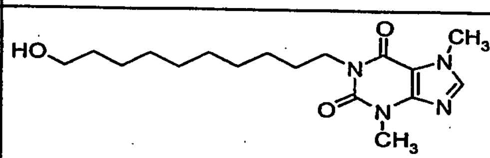
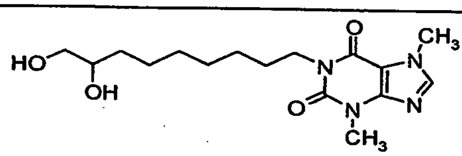
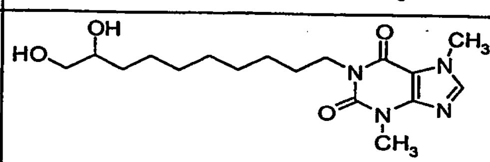
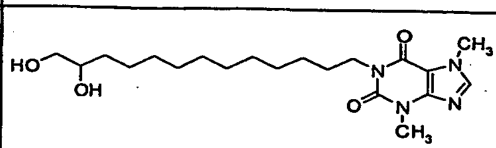
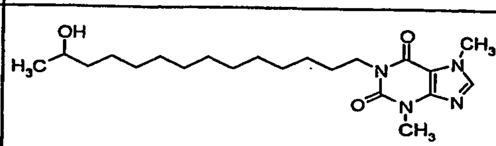
968		Allergan		US 5013850
969		Allergan		US 5013850
970		Allergan		US 5013850
971		Allergan		US 5013850
972		Allergan		US 5013850
973		Allergan		US 5013850
974		Allergan		US 5013850
975		Allergan		US 5013850
976		Allergan		US 5013850
977		Allergan		US 5013850
978		Allergan		US 5013850

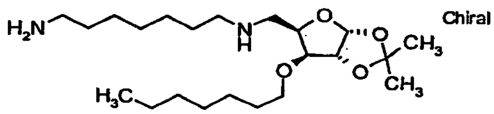
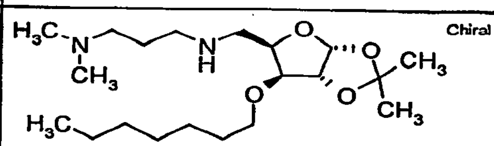
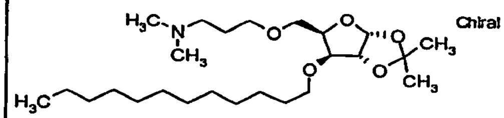
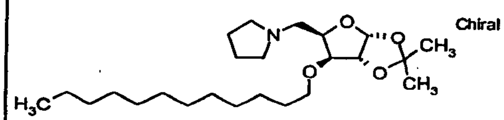
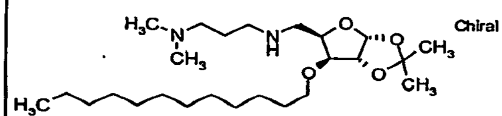
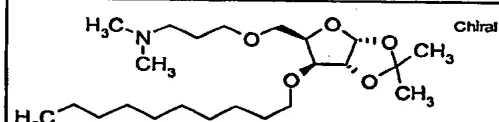
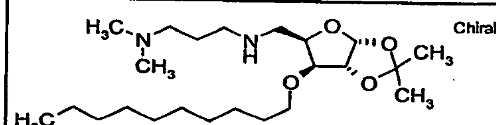
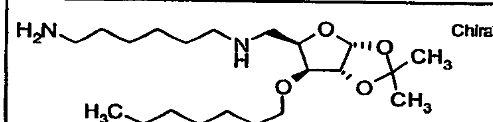
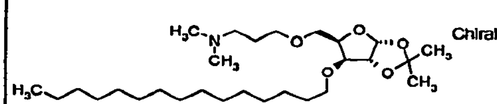
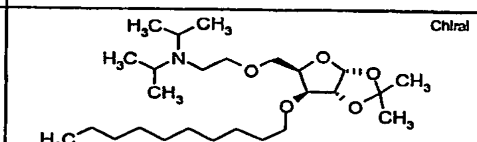
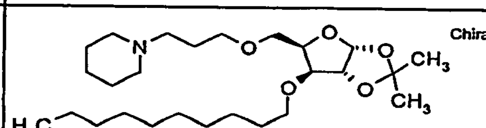
979		New England Med. Center Hosp.		WO 9116339
980		Roche	1) Hill, C.H. 6th SCI-RSC Med Chem Symp (Sept 8-11, Cambridge) 1991, Abst S18 .	EP 384349
981		New England Med. Center Hosp.		WO 9116339
982		Alder		US 5145842
983		Alder		US 5145842
984		Alder		US 5145842
985		Alder		US 5145842
986		Alder		US 5145842
987		Alder		US 5145842
988		Alder		US 5145842
989		Alder		US 5145842

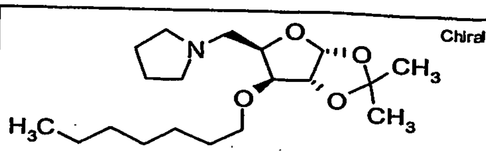
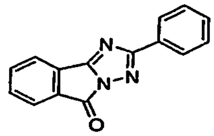
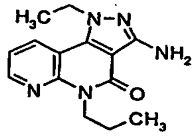
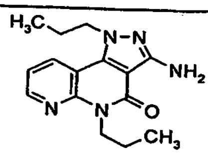
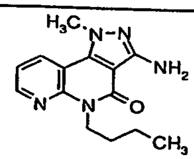
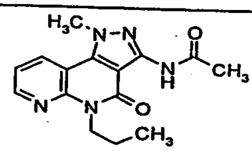
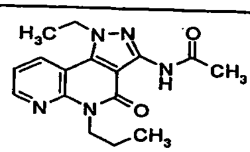
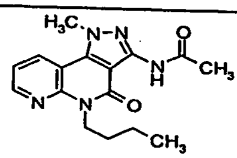
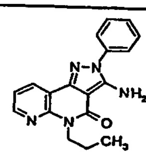
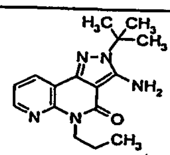
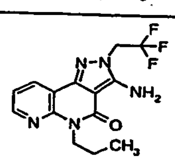
990		Roche		EP 510473
991		Beaufour-Ipsen	1) Carde, P. et al. Proc Amer Soc Clin Oncol 1991, 10: Abst 324.	AU 8810261
992		Roche		EP 510473
993		Roche		EP 510473
994		Roche		EP 510473
995		Fujisawa		WO 9218483
996		Kyowa Hakko		EP 526840
997		Kyowa Hakko		EP 526840
998		Kyowa Hakko		EP 526840
999		Aventis Pharma		EP 538783
1000		Hayashibara	1) Yamamoto, I. et al. 18th Int Cong Chemother (June 27-July 2, Stockholm) 1993, Abst 516 .	EP 539196

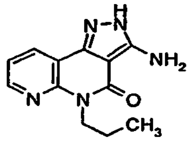
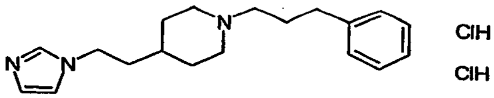
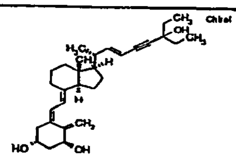
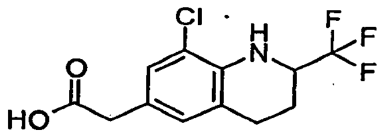
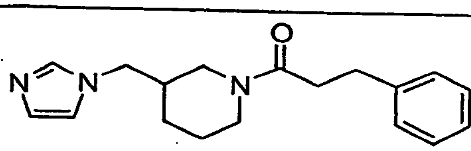
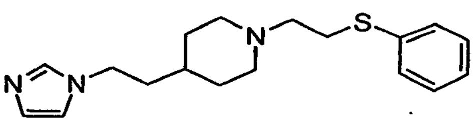
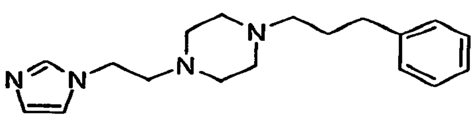
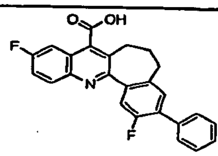
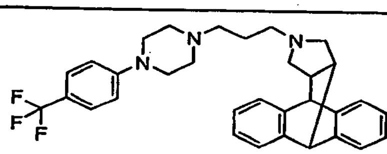
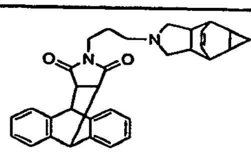
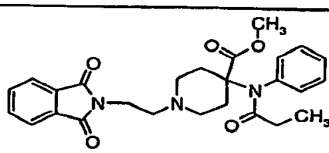
1001		Kyowa Hakko		WO 9312116
1002		GlaxoSmithKline		WO 9314082
1003		Otsuka		JP 93132484
1004		Pfizer		US 5236926
1005		Aventis Pharma		EP 538783
1006		Aventis Pharma		EP 538783
1007		GlaxoSmithKline		WO 9314082
1008		GlaxoSmithKline		WO 9314082
1009		GlaxoSmithKline		WO 9314082
1010		GlaxoSmithKline		WO 9314082
1011		GlaxoSmithKline		WO 9314082

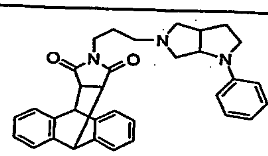
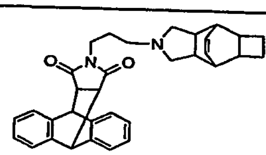
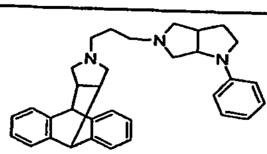
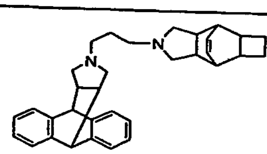
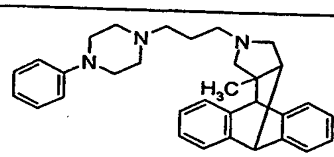
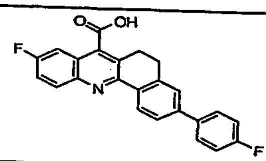
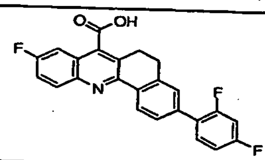
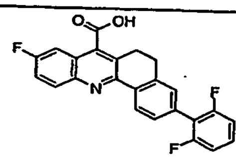
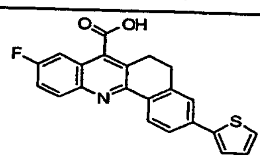
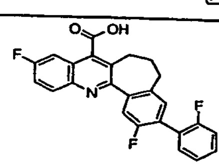
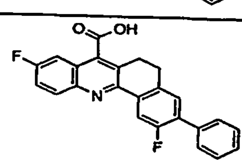
1012		GlaxoSmithKline		WO 9314082
1013		Otsuka		JP 93132484
1014		Otsuka		JP 93132484
1015		Otsuka		JP 93132484
1016		Otsuka		JP 93132484
1017		Otsuka		JP 93132484
1018		Pfizer		US 5236926
1019		Pfizer		US 5236926
1020		Pfizer		US 5236926
1021		Pfizer		US 5236926
1022		Pfizer		US 5236926

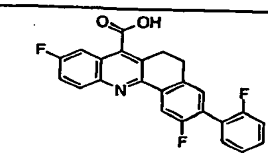
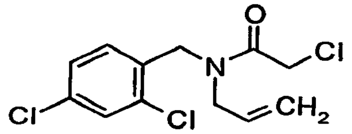
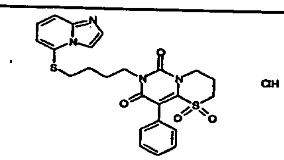
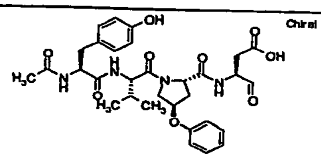
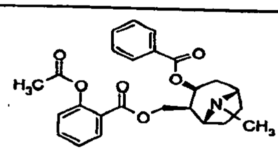
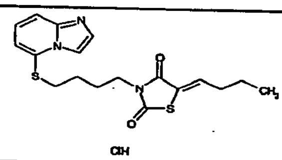
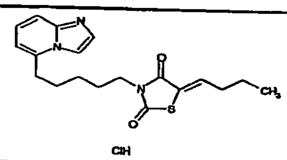
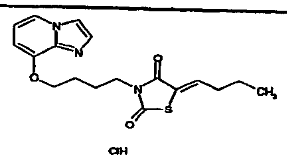
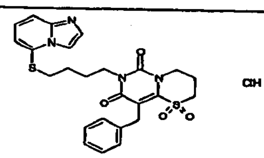
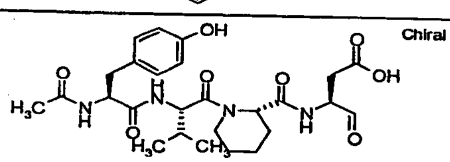
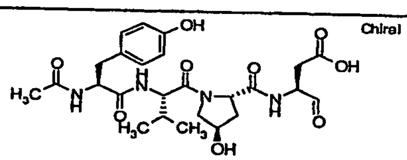
1023		Sumitomo		US 5258396
1024		Cell Therapeutics		WO 9411001
1025	 Chiral	Greenwich Pharm.		WO 9411381
1026		Otsuka		JP 94100561
1027		Cell Therapeutics		WO 9411001
1028		Cell Therapeutics		WO 9411001
1029		Cell Therapeutics		WO 9411001
1030		Cell Therapeutics		WO 9411001
1031		Cell Therapeutics		WO 9411001
1032		Cell Therapeutics		WO 9411001
1033		Cell Therapeutics		WO 9411001

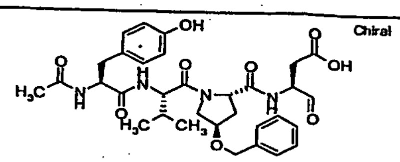
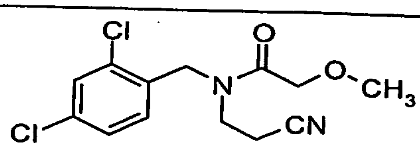
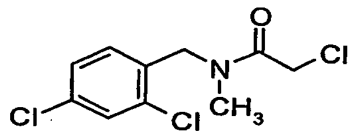
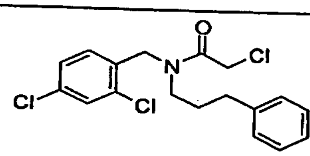
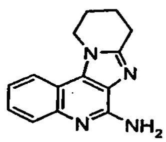
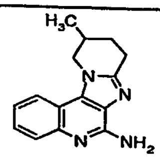
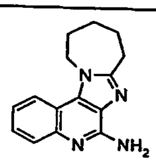
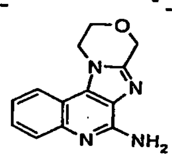
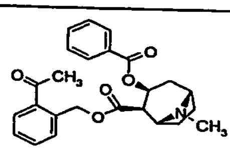
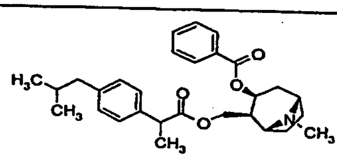
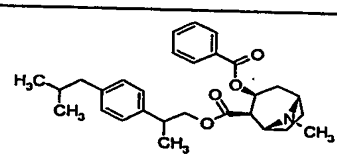
1034		Greenwich Pharm.		WO 9411381
1035		Greenwich Pharm.		WO 9411381
1036		Greenwich Pharm.		WO 9411381
1037		Greenwich Pharm.		WO 9411381
1038		Greenwich Pharm.		WO 9411381
1039		Greenwich Pharm.		WO 9411381
1040		Greenwich Pharm.		WO 9411381
1041		Greenwich Pharm.		WO 9411381
1042		Greenwich Pharm.		WO 9411381
1043		Greenwich Pharm.		WO 9411381
1044		Greenwich Pharm.		WO 9411381

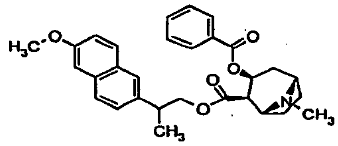
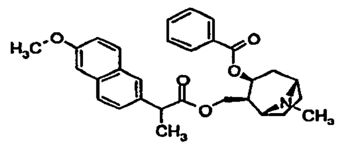
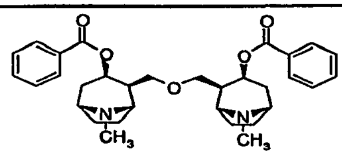
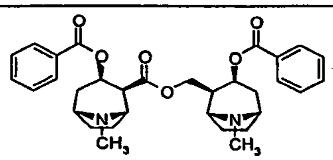
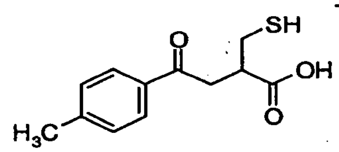
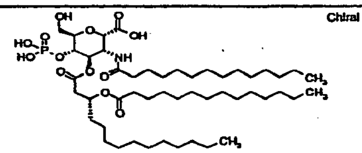
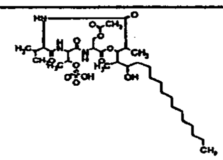
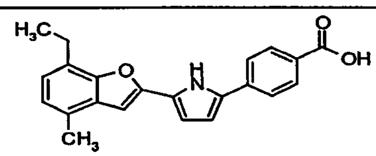
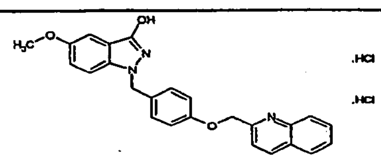
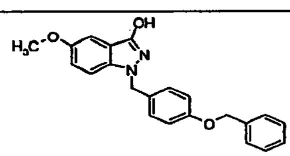
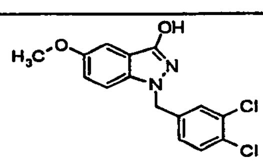
1045		Greenwich Pharm.		WO 9411381
1046		British Technol.		GB 2278842
1047		Otsuka		JP 94100561
1048		Otsuka		JP 94100561
1049		Otsuka		JP 94100561
1050		Otsuka		JP 94100561
1051		Otsuka		JP 94100561
1052		Otsuka		JP 94100561
1053		Otsuka		JP 94100561
1054		Otsuka		JP 94100561
1055		Otsuka		JP 94100561

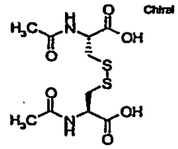
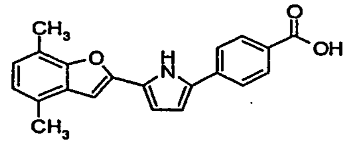
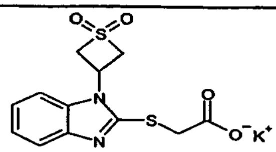
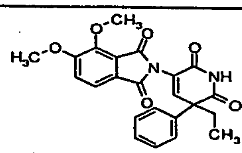
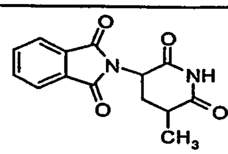
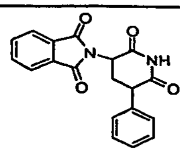
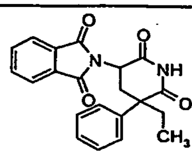
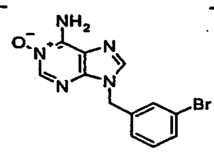
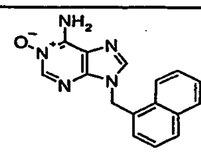
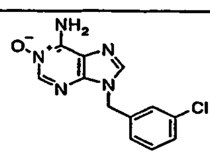
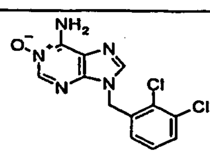
1056		Otsuka		JP 94100561
1057		Abbott GmbH		WO 9500507
1058		Leo		WO 9502577
1059		Kyorin	Kono, Y. et al. 115th Annu Meet Pharmaceut Soc Jpn (March 29-31, Sendai) 1995, Abst 30 (A3) 10-3 .	EP 538477
1060		Abbott GmbH		WO 9500507
1061		Abbott GmbH		WO 9500507
1062		Abbott GmbH		WO 9500507
1063		Kyowa Hakko		WO 9509153
1064		Bayer		US 5409932
1065		Bayer		US 5411960
1066		Baxter	France, C.P. et al. Drug Develop Res 1995, 35: 49.	EP 396282

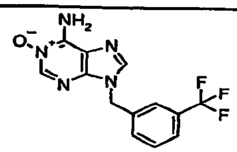
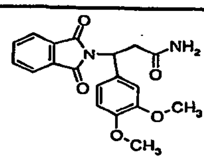
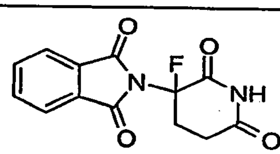
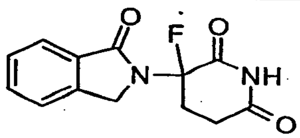
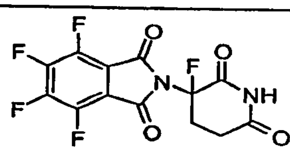
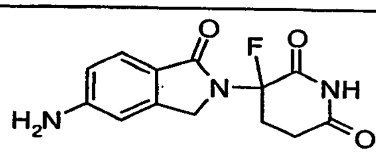
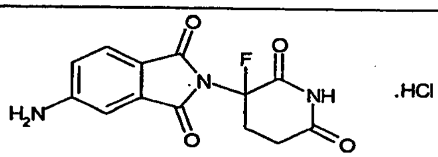
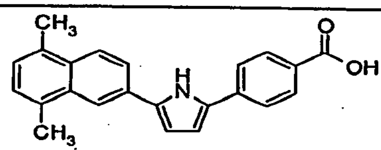
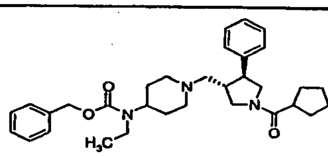
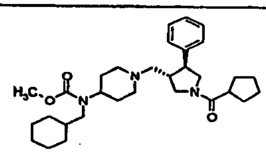
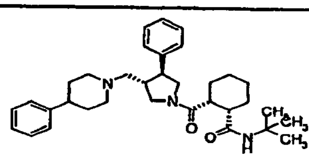
1067		Bayer		US 5411960
1068		Bayer		US 5411960
1069		Bayer		US 5411960
1070		Bayer		US 5411960
1071		Bayer		US 5409932
1072		Kyowa Hakko		WO 9509153
1073		Kyowa Hakko		WO 9509153
1074		Kyowa Hakko		WO 9509153
1075		Kyowa Hakko		WO 9509153
1076		Kyowa Hakko		WO 9509153
1077		Kyowa Hakko		WO 9509153

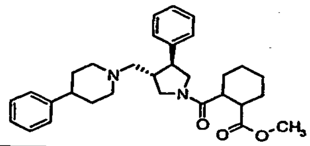
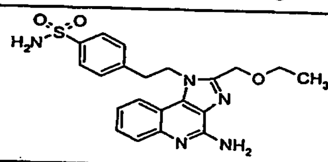
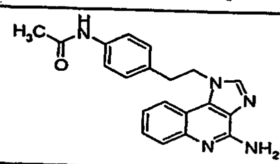
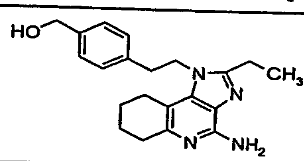
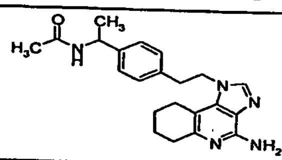
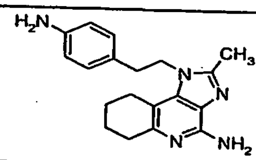
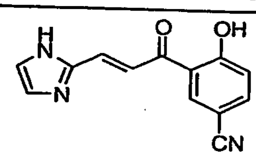
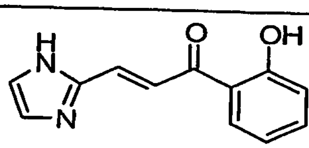
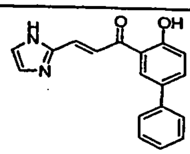
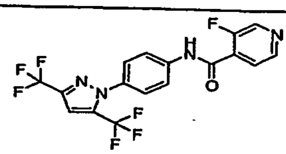
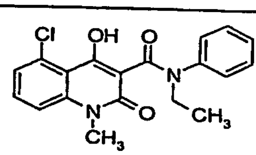
1078		Kyowa Hakko		WO 9509153
1079		Sanofi-Synthelabo		WO 9529672
1080		Takeda		WO 9535296
1081		Vertex		WO 9535308
1082		Entropin		WO 9534561
1083		Takeda		WO 9535296
1084		Takeda		WO 9535296
1085		Takeda		WO 9535296
1086		Takeda		WO 9535296
1087		Vertex		WO 9535308
1088		Vertex		WO 9535308

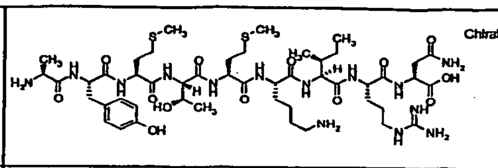
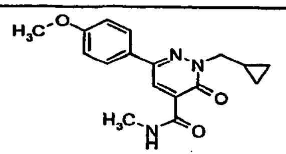
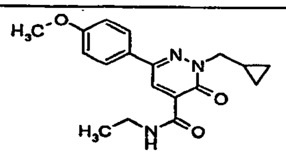
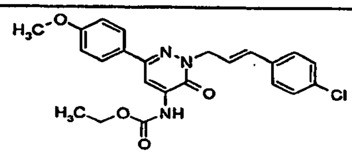
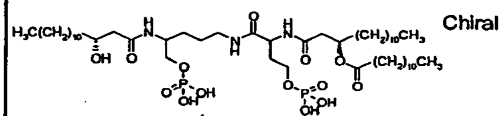
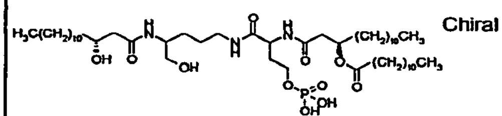
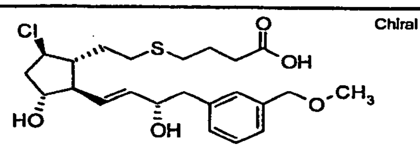
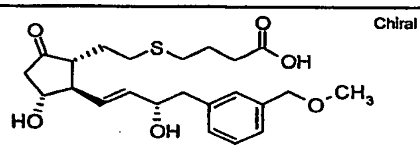
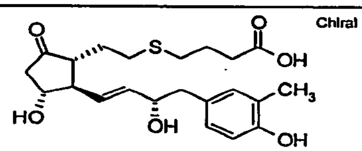
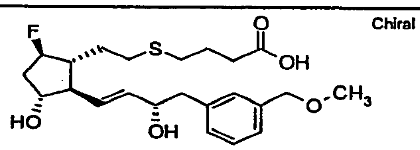
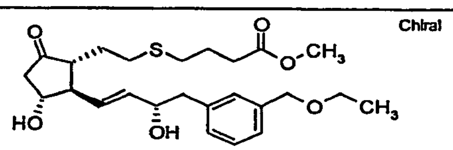
1089		Vertex		WO 9535308
1090		Sanofi-Synthelabo		WO 9529672
1091		Sanofi-Synthelabo		WO 9529672
1092		Sanofi-Synthelabo		WO 9529672
1093		3M Pharmaceuticals		US 5482936
1094		3M Pharmaceuticals		US 5482936
1095		3M Pharmaceuticals		US 5482936
1096		3M Pharmaceuticals		US 5482936
1097		Entropin		WO 9534561
1098		Entropin		WO 9534561
1099		Entropin		WO 9534561

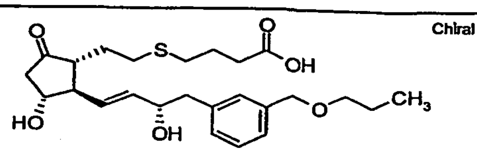
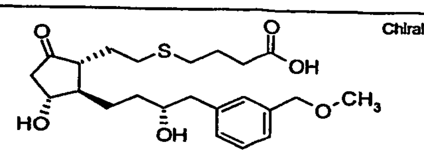
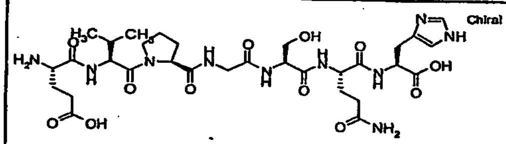
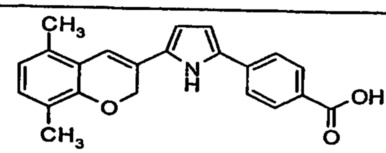
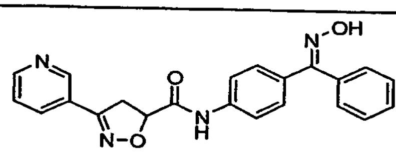
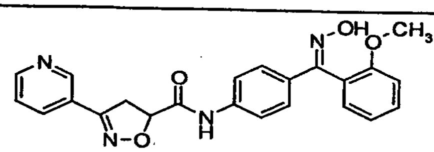
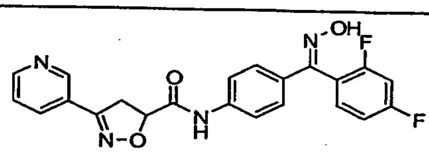
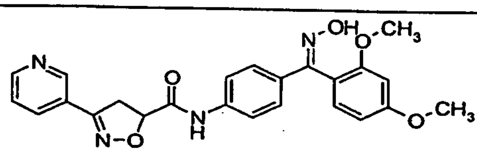
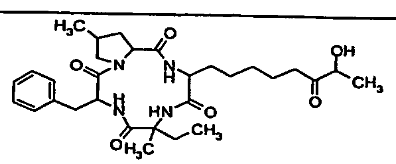
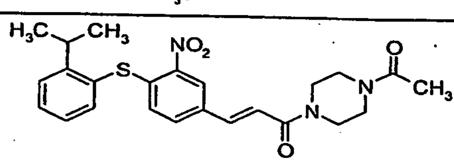
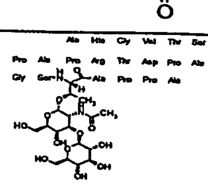
1100		Entropin		WO 9534561
1101		Entropin		WO 9534561
1102		Entropin		WO 9534561
1103		Entropin		WO 9534561
1104		Taisho	1) Yoshida, H. et al. Biol Pharm Bull 1997, 20(1): 94.	JP 93051358
1105		Sankyo	Shiozaki, M. et al. Tetrahedron Lett 1996 37(40): 7271.	
1106		Nippon Kayaku		JP 96283290
1107		Eisai		EP 889032
1108		AWD.pharma		WO 9734874
1109		AWD.pharma		WO 9734874
1110		AWD.pharma		WO 9734874

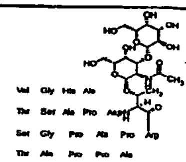
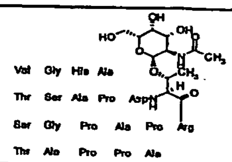
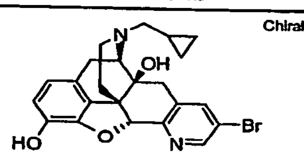
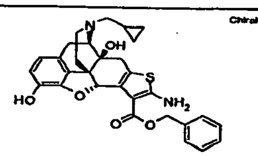
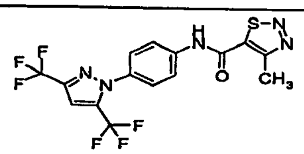
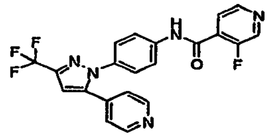
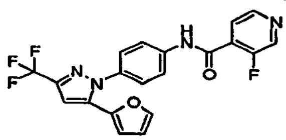
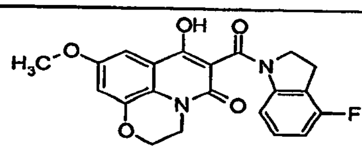
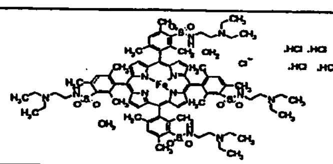
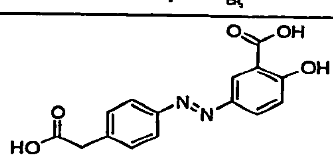
1111		AstraZeneca	1) Saemstrand, B. et al. J Pharmacol Exp Ther 1999, 288(3): 1174.	EP 0463514
1112		Eisai	Nagai, M. et al. 217th ACS Natl Meet (March 21-25, Anaheim) 1999, Abst MEDI 050.	EP 889032
1113		Bashkir Medical University	Sadykov, R.F. et al. Naunyn-Schmied Arch Pharmacol 1998, 358(1, Suppl. 2): Abst P 52.28.	
1114		Gruenenthal		EP 856513
1115		Gruenenthal		EP 856513
1116		Gruenenthal		EP 856513
1117		Gruenenthal		EP 856513
1118		Japan Energy		JP 98231297
1119		Japan Energy		JP 98231297
1120		Japan Energy		JP 98231297
1121		Japan Energy		JP 98231297

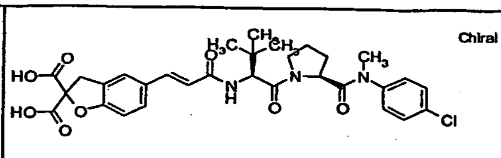
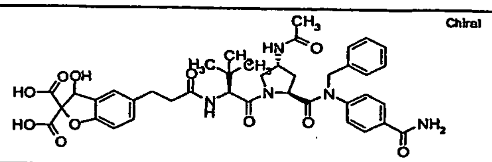
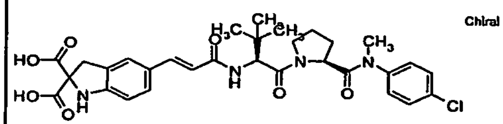
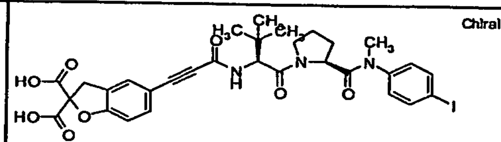
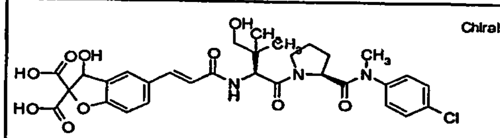
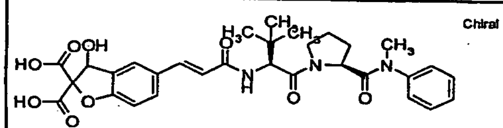
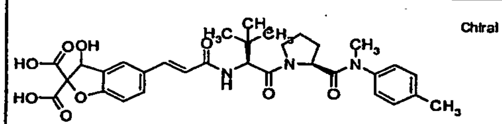
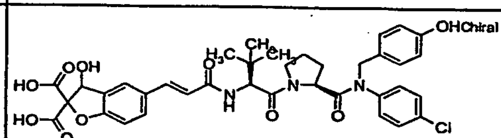
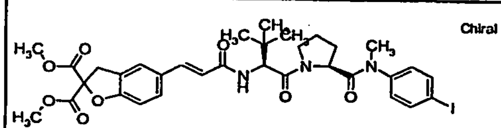
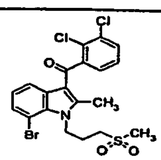
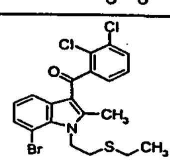
1122		Japan Energy		JP 98231297
1123		Celgene	1) Moreira, A.L. et al. J Neuro-Oncol 1999, 43(2): 109.	US 5463063
1124		Celgene		US 5874448
1125		Celgene		US 5874448
1126		Celgene		US 5874448
1127		Celgene		US 5874448
1128		Celgene		US 5874448
1129		Eisai		EP 889032
1130		Merck & Co.		WO 9909984
1131		Merck & Co.		WO 9909984
1132		Merck & Co.		WO 9909984

1133		Merck & Co.		WO 9909984
1134		Hokuriku		JP 99080156
1135		Hokuriku		JP 99080156
1136		Hokuriku		JP 99080156
1137		Hokuriku		JP 99080156
1138		Hokuriku		JP 99080156
1139		SSP		CA 2255337
1140		SSP		CA 2255337
1141		SSP		CA 2255337
1142		Abbott	Madar, D. et al. 222nd ACS Natl Meet (Aug 26-30, Chicago) 2001, Abst MEDI 7.	EP 1068187
1143		Active Biotech		WO 9955678

1144		Amersham	1) Gesser, B. et al. Proc Natl Acad Sci USA 1997, 94(26): 14620.	WO 9601318
1145		Kowa		WO 9944995
1146		Kowa		WO 9944995
1147		Kowa		WO 9944995
1148		OM Pharma		WO 0000462
1149		OM Pharma		WO 0000462
1150		Ono		WO 0003980
1151		Ono		WO 0003980
1152		Ono		WO 0003980
1153		Ono		WO 0003980
1154		Ono		WO 0003980

1155		Ono		WO 0003980
1156		Ono		WO 0003980
1157		University of Bristol		WO 0014114
1158		Eisai	Hibi, S. et al. Bioorg Med Chem Lett 2000, 10(7): 623.	EP 0889032
1159		Janssen		WO 0021959
1160		Janssen		WO 0021959
1161		Janssen		WO 0021959
1162		Janssen		WO 0021959
1163		Fujisawa		WO 0021979
1164		Abbott	1) Liu, G. et al. 220th ACS Natl Meet (Aug 20-24, Washington DC) 2000, Abst MEDI 171.	US 6110922
1165		Cancer Research UK		WO 0052046

1166		Cancer Research UK		WO 0052046
1167		Cancer Research UK		WO 0052046
1168		Southern Research Institute		WO 0112197
1169		Southern Research Institute		WO 0112197
1170		Abbott Labs.		EP 1068187
1171		Abbott		EP 1068187
1172		Abbott		EP 1068187
1173		Fujisawa	Spears, G. et al. 21st Symp Med Chem (Nov 28-30, Kyoto) 2001, Abst 2P-29.	
1174	Val His Phe Phe Arg Asn Ile Pro Thr Arg Ala Thr Val	Austin Research Institute	Tselios, T. et al. J Med Chem 2002, 45(2): 275.	
1175		Eukarion		WO 0204454
1176		Nobex	1) Riggs-Sauthier, J.A. et al. 224th ACS Natl Meet (Aug 18-22, Boston) 2002, Abst MEDI 305.	WO 0218324

1177		Tularik		WO 0238107
1178		Tularik		WO 0238107
1179		Tularik		WO 0238107
1180		Tularik		WO 0238107
1181		Tularik		WO 0238107
1182		Tularik		WO 0238107
1183		Tularik		WO 0238107
1184		Tularik		WO 0238107
1185		Tularik		WO 0238107
1186		Sanofi-Synthelabo		WO 0242269
1187		Sanofi-Synthelabo		WO 0242269

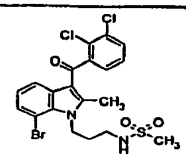
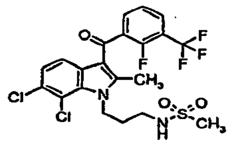
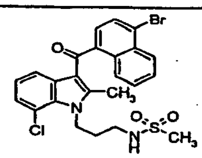
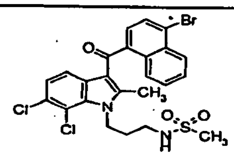
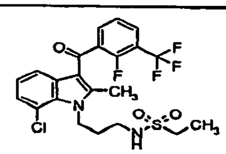
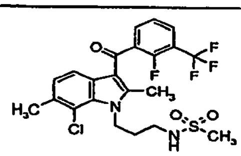
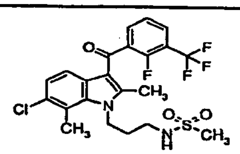
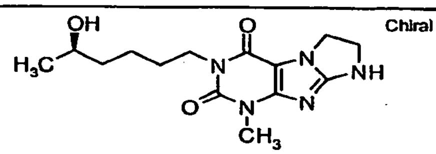
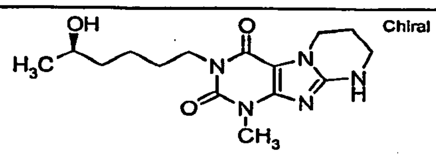
1188		Sanofi-Synthelabo		WO 0242269
1189		Sanofi-Synthelabo		WO 0242269
1190		Sanofi-Synthelabo		WO 0242269
1191		Sanofi-Synthelabo		WO 0242269
1192		Sanofi-Synthelabo		WO 0242269
1193		Sanofi-Synthelabo		WO 0242269
1194		Sanofi-Synthelabo		WO 0242269
1195		Cell Therapeutics		WO 0268421
1196		Cell Therapeutics		WO 0268421

Table 35 Continued

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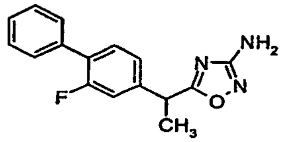
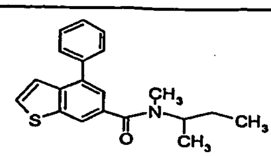
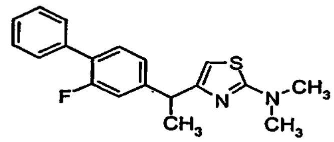
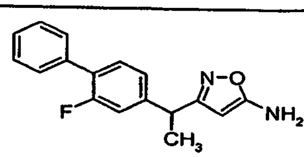
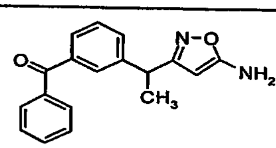
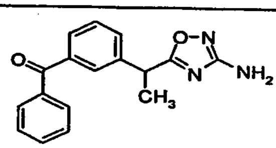
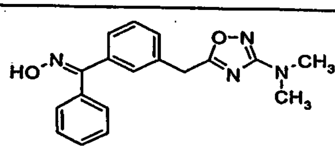
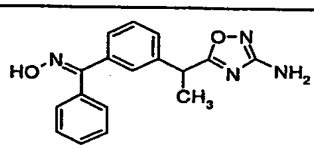
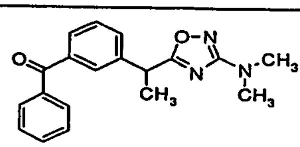
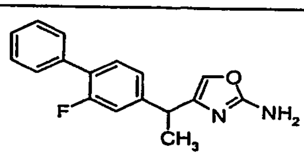
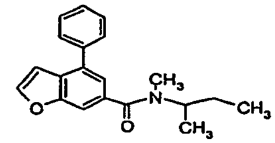
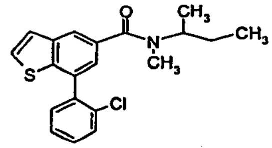
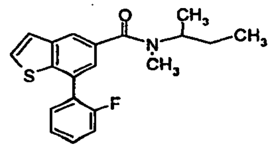
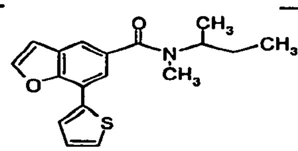
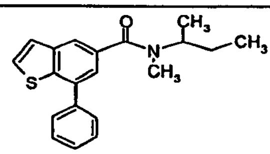
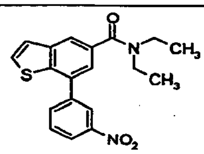
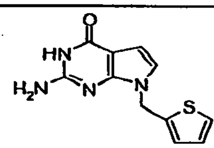
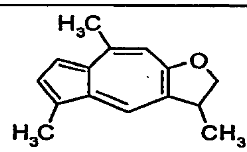
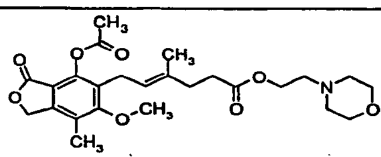
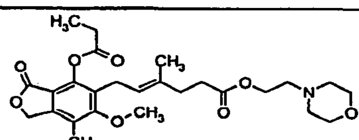
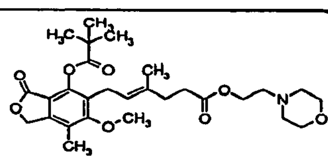
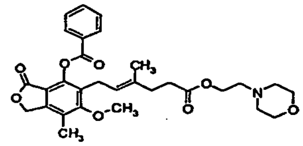
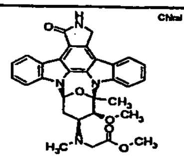
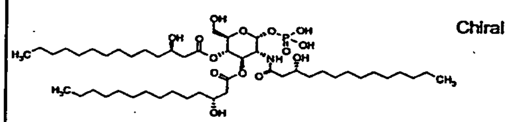
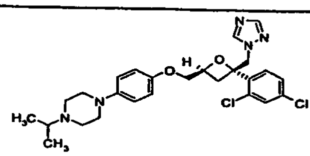
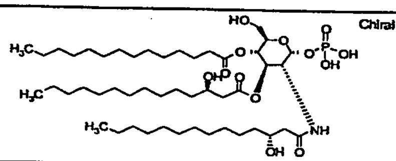
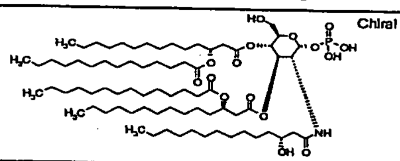
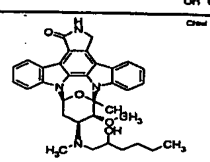
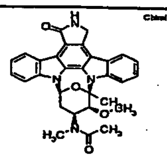
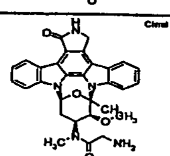
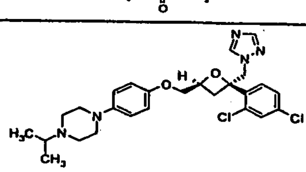
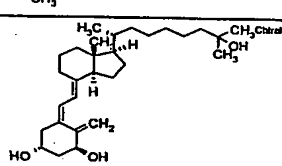
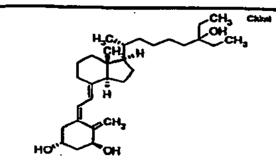
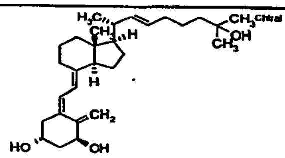
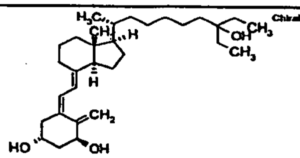
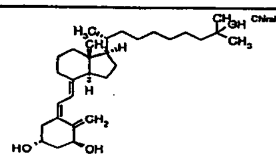
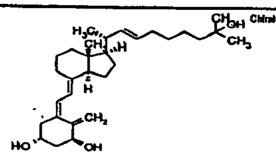
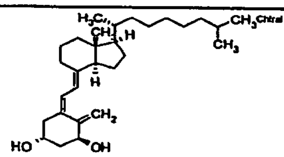
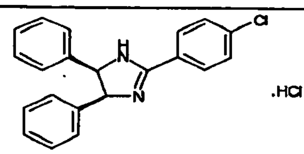
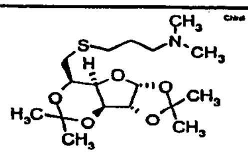
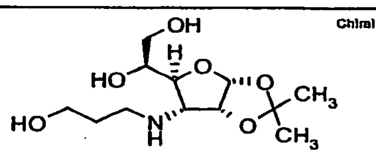
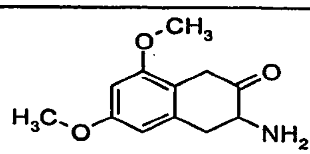
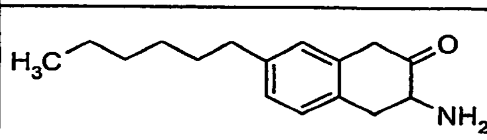
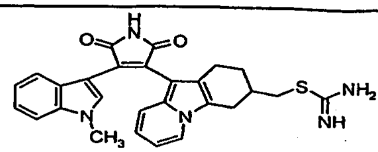
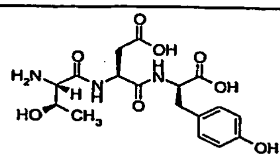
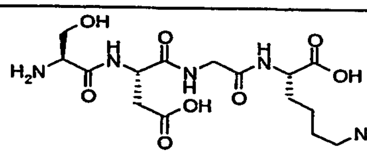
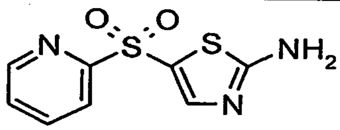
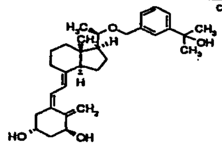
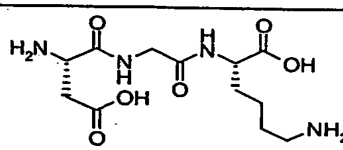
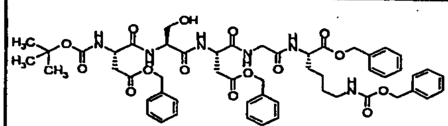
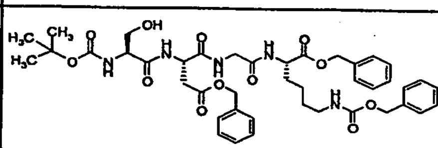
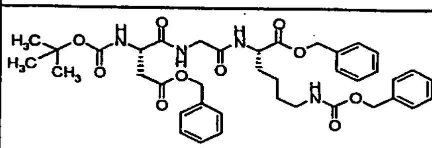
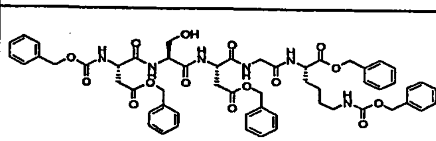
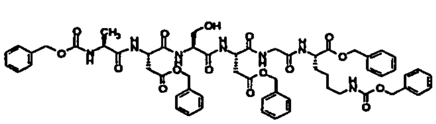
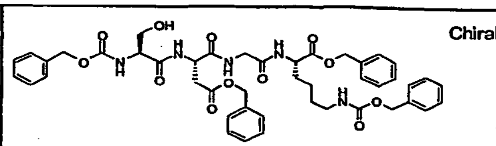
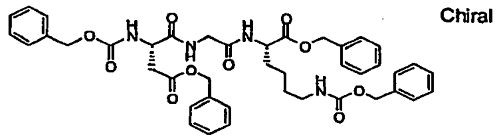
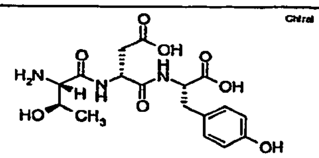
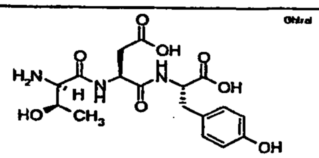
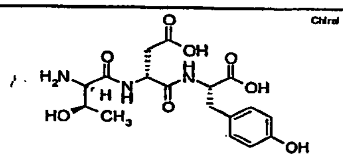
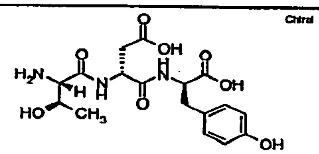
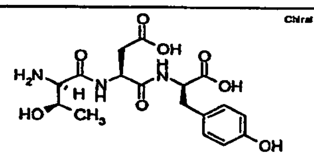
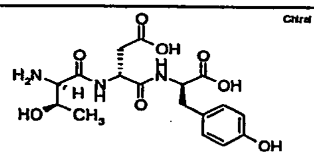
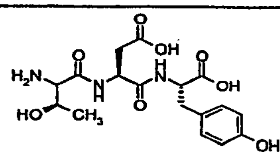
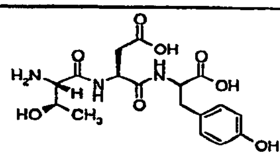
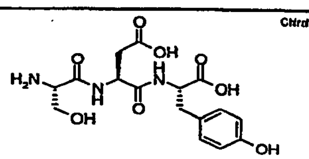
1197		Sumitomo Pharmaceuticals		EP 248399
1198		Aventis Pharma		EP 248734
1199		Sumitomo Pharmaceuticals		EP 248399
1200		Sumitomo Pharmaceuticals		EP 248399
1201		Sumitomo Pharmaceuticals		EP 248399
1202		Sumitomo Pharmaceuticals		EP 248399
1203		Sumitomo Pharmaceuticals		EP 248399
1204		Sumitomo Pharmaceuticals		EP 248399
1205		Sumitomo Pharmaceuticals		EP 248399
1206		Sumitomo Pharmaceuticals		EP 248399

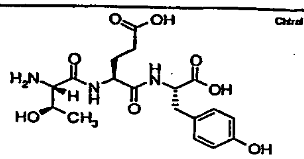
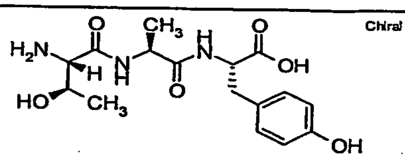
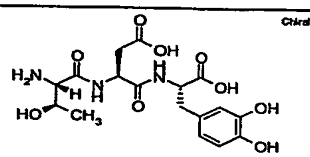
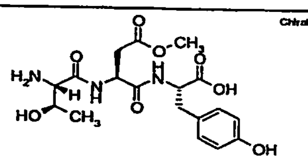
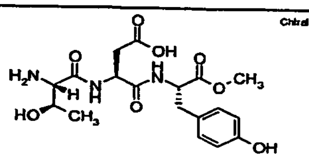
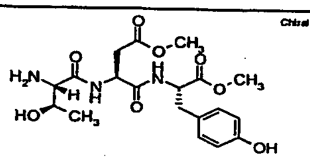
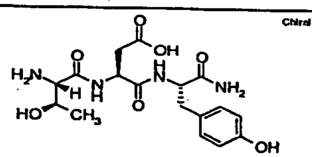
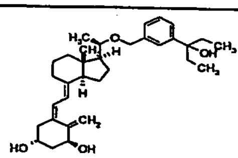
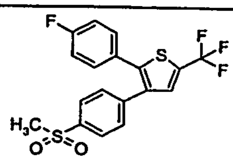
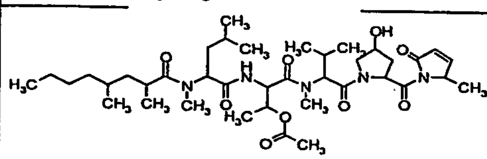
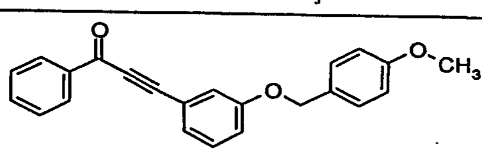
1207		Aventis Pharma		EP 248734
1208		Aventis Pharma		EP 248734
1209		Aventis Pharma		EP 248734
1210		Aventis Pharma		EP 248734
1211		Aventis Pharma		EP 248734
1212		Aventis Pharma		EP 248734
1213		Pfizer		AU 8783281
1214		Harbor Branch Found.		US 4755529
1215		Roche Bioscience		AU 8782540
1216		Roche Bioscience		AU 8782540
1217		Roche Bioscience		AU 8782540

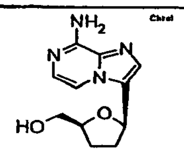
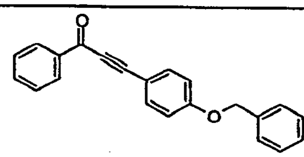
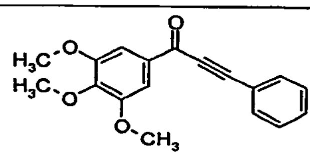
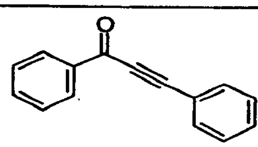
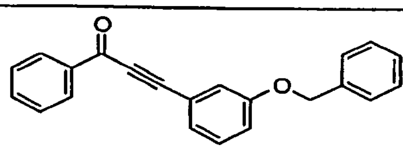
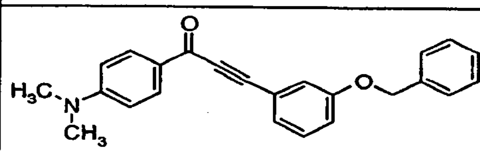
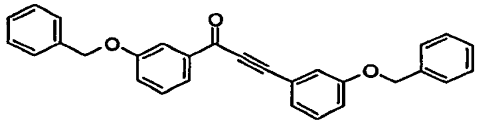
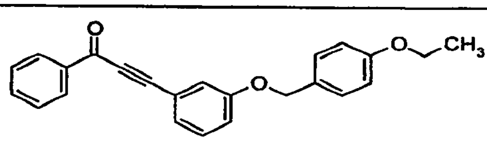
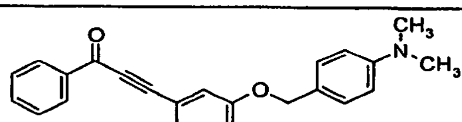
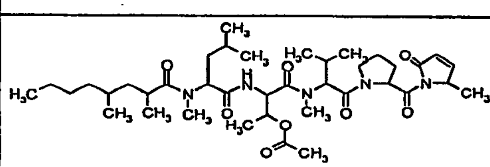
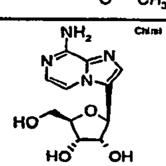
1218		Roche Bioscience		AU 8782540
1219		Novartis		EP 296110
1220		Novartis	1) Lam, C. et al. Antimicrob Agents Chemother 1991, 35(3): 500.	AU 8822785
1221		Schering-Plough		EP 318214
1222		Novartis		AU 8822785
1223		Novartis		AU 8822785
1224		Novartis		EP 296110
1225		Novartis		EP 296110
1226		Novartis		EP 296110
1227		Schering-Plough		EP 318214
1228		Leo		WO 8910351

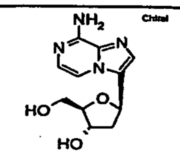
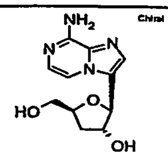
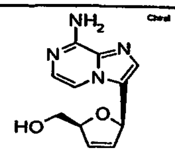
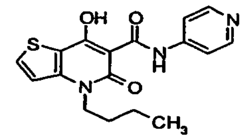
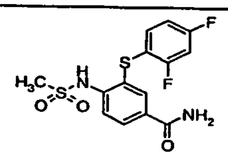
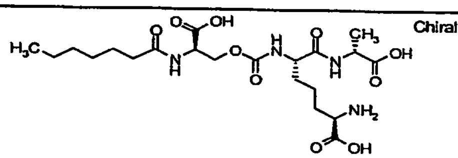
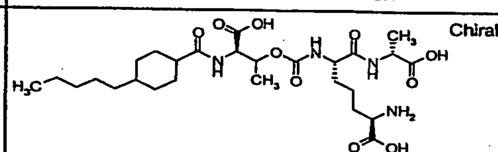
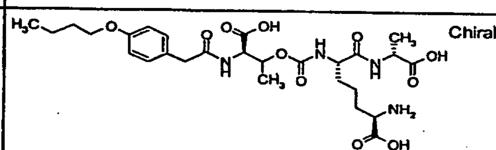
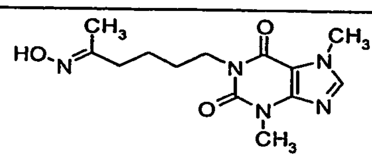
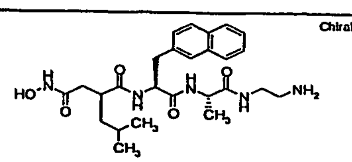
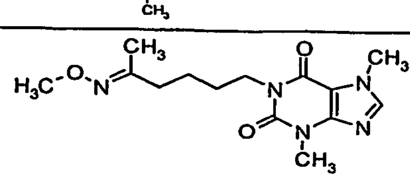
1229		Leo		WO 8910351
1230		Leo		WO 8910351
1231		Leo		WO 8910351
1232		Leo		WO 8910351
1233		Leo		WO 8910351
1234		Leo		WO 8910351
1235		Tanabe Seiyaku	1) Ueno, M. et al. Jpn J Pharmacol 1992, 58(Suppl. 1): Abst O-210.	AU 8942368
1236		Greenwich Pharm.		AU 9047648
1237		Greenwich Pharm.		AU 9047648
1238		Aventis Pharma		EP 378456
1239		Aventis Pharma		EP 378456

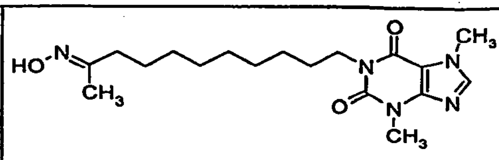
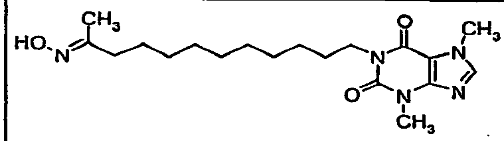
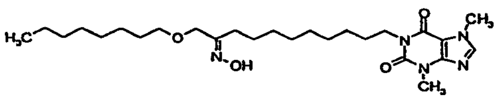
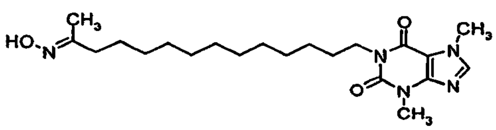
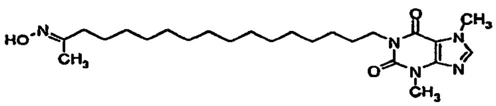
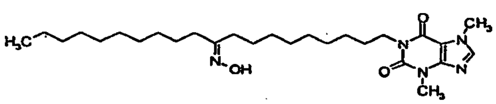
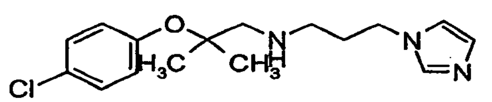
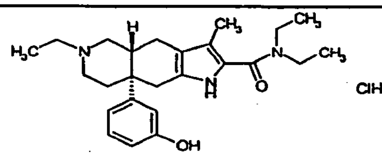
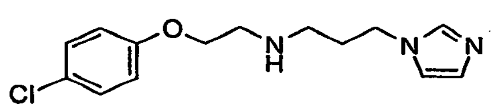
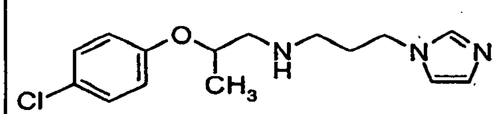
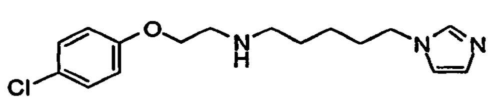
1240		Roche		EP 384349
1241	 Chiral	SPA		EP 421074
1242	 Chiral	Hitachi Chemical		EP 421682
1243		Fujisawa	1) Manda, T. et al. Jpn J Pharmacol 1997, 73(Suppl. 1): Abst P-140.	EP 412404
1244	 Chiral	Leo		WO 9109841
1245	 Chiral	Hitachi Chemical		EP 421682
1246	 Chiral	Hitachi Chemical		EP 421682
1247	 Chiral	Hitachi Chemical		EP 421682
1248	 Chiral	Hitachi Chemical		EP 421682
1249	 Chiral	Hitachi Chemical		EP 421682
1250	 Chiral	Hitachi Chemical		EP 421682

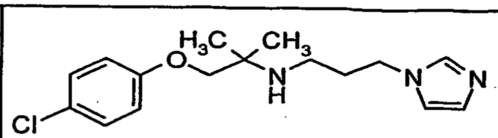
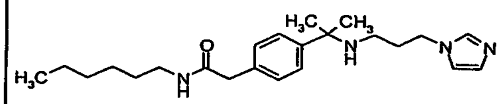
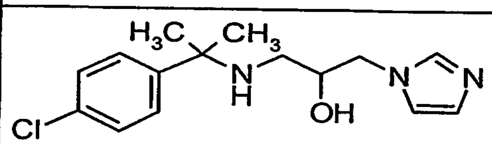
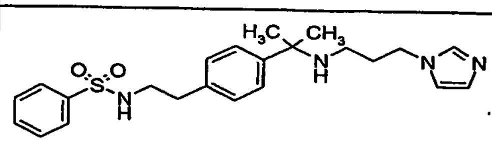
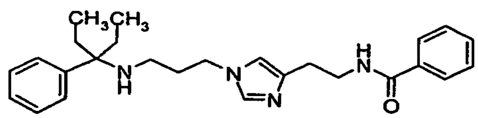
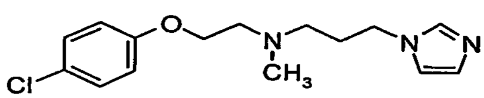
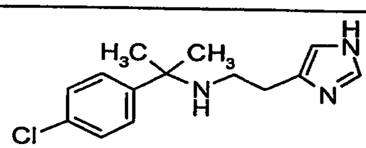
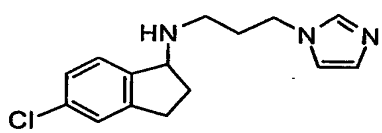
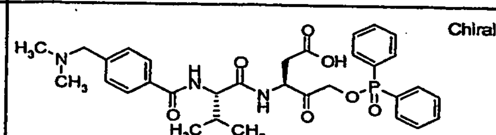
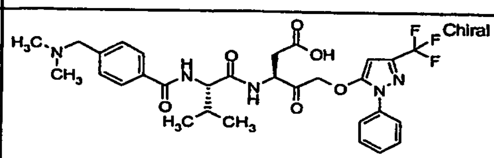
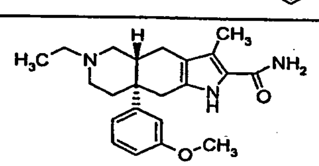
1251		Hitachi Chemical		EP 421682
1252		Hitachi Chemical		EP 421682
1253		SPA		EP 421074
1254		SPA		EP 421074
1255		SPA		EP 421074
1256		SPA		EP 421074
1257		SPA		EP 421074
1258		SPA		EP 421074
1259		SPA		EP 421074
1260		SPA		EP 421074
1261		SPA		EP 421074

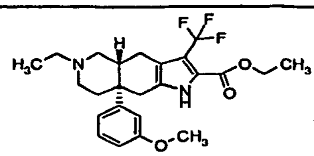
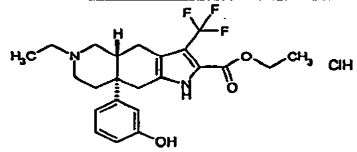
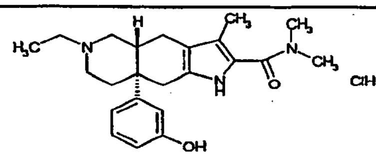
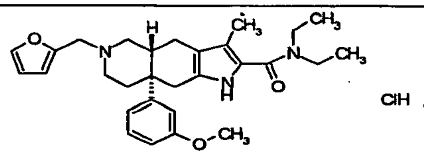
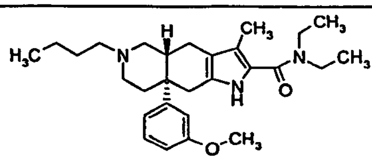
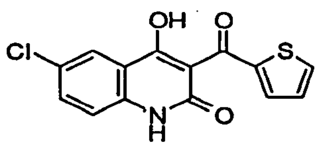
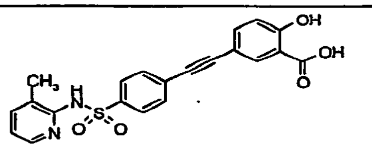
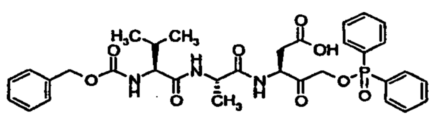
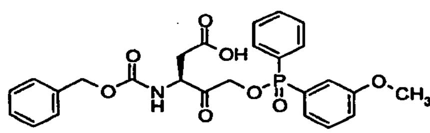
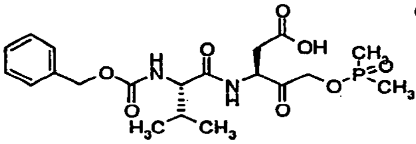
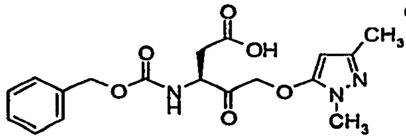
1262		SPA		EP 421074
1263		SPA		EP 421074
1264		SPA		EP 421074
1265		SPA		EP 421074
1266		SPA		EP 421074
1267		SPA		EP 421074
1268		SPA		EP 421074
1269		Leo		EP 506794
1270		Fujisawa		WO 9119708
1271		Harbor Branch Found.		US 5091368
1272		Aventis Pharma		EP 476658

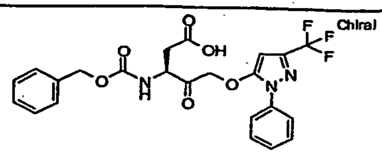
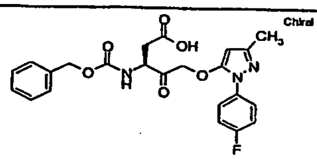
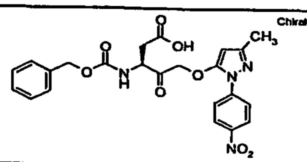
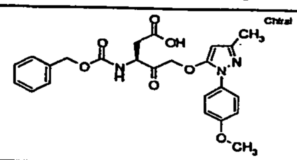
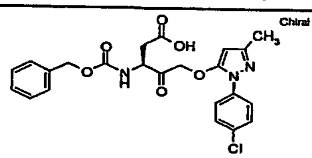
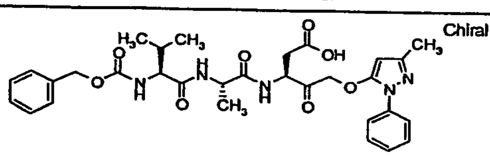
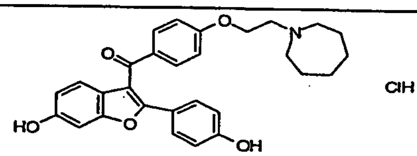
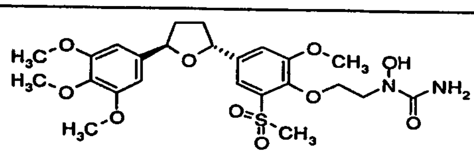
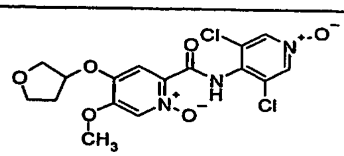
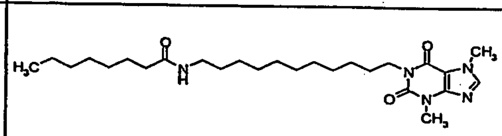
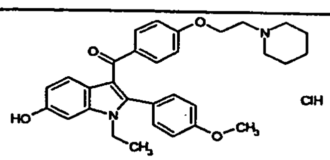
1273		Merck & Co.		EP 480713
1274		Aventis Pharma		EP 476658
1275		Aventis Pharma		EP 476658
1276		Aventis Pharma		EP 476658
1277		Aventis Pharma		EP 476658
1278		Aventis Pharma		EP 476658
1279		Aventis Pharma		EP 476658
1280		Aventis Pharma		EP 476658
1281		Aventis Pharma		EP 476658
1282		Harbor Branch Found.		US 5091368
1283		Merck & Co.		EP 480713

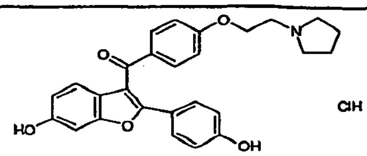
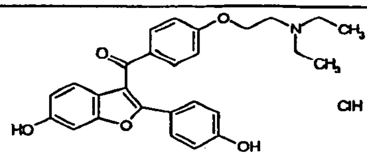
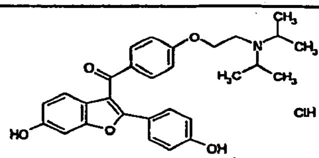
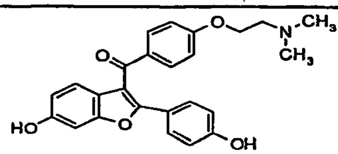
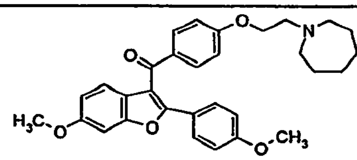
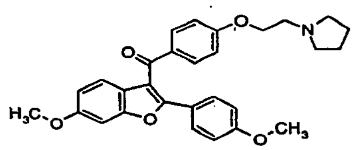
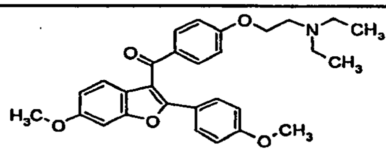
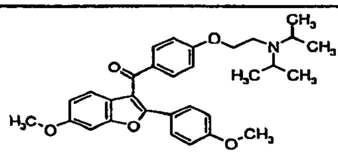
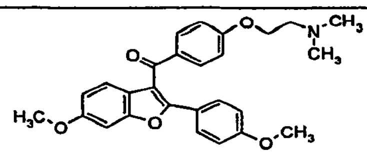
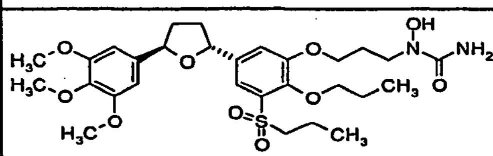
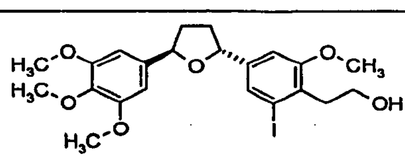
1284		Merck & Co.		EP 480713
1285		Merck & Co.		EP 480713
1286		Merck & Co.		EP 480713
1287		Kyowa Hakko	Miwa, K. et al. 113th Annu Meet Pharmaceut Soc Jpn (March 29-31, Osaka) 1993, Abst 30CC 13-1 .	EP 505058
1288		Fujisawa	Nakamura, K. et al. Chem Pharm Bull 1993, 41(5): 894.	AU 8783152
1289		Wyeth		US 5312831
1290		Wyeth		US 5312831
1291		Wyeth		US 5312831
1292		Cell Therapeutics		WO 9416704
1293		Immunex	1) Immunex Corporation Press Release 1994, July 21.	WO 9506031
1294		Cell Therapeutics		WO 9416704

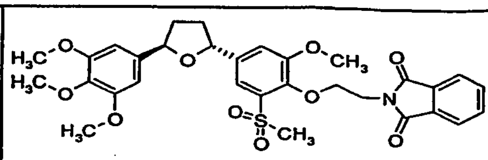
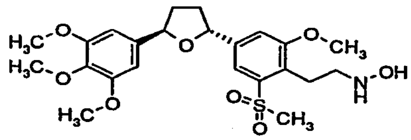
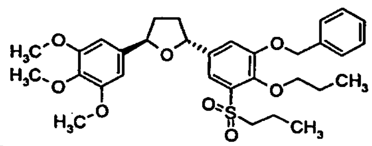
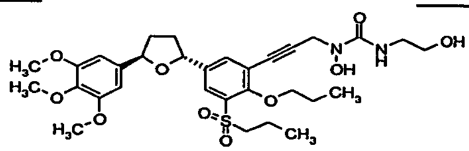
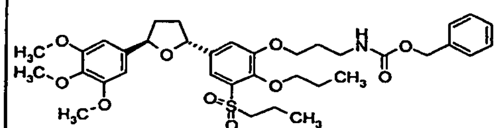
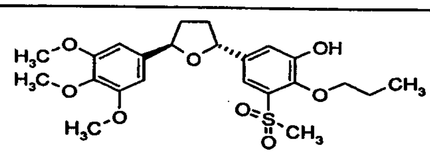
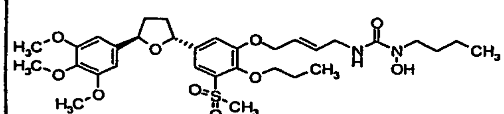
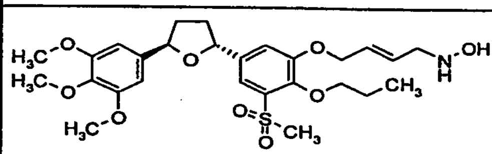
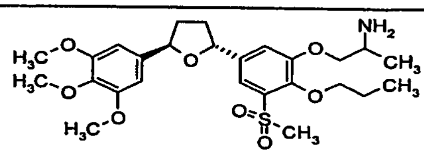
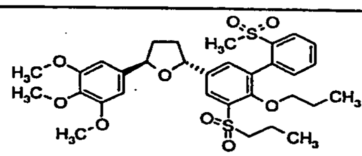
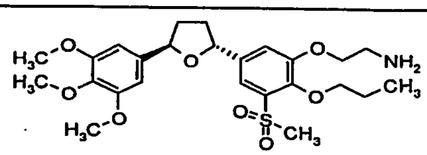
1295		Cell Therapeutics		WO 9416704
1296		Cell Therapeutics		WO 9416704
1297		Cell Therapeutics		WO 9416704
1298		Cell Therapeutics		WO 9416704
1299		Cell Therapeutics		WO 9416704
1300		Cell Therapeutics		WO 9416704
1301		Abbott GmbH		WO 9500493
1302		GlaxoSmithKline		WO 9504734
1303		Abbott GmbH		WO 9500493
1304		Abbott GmbH		WO 9500493
1305		Abbott GmbH		WO 9500493

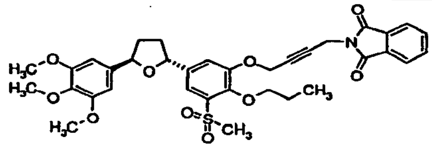
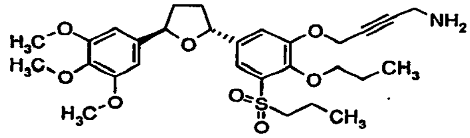
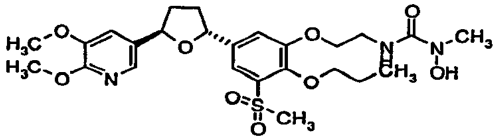
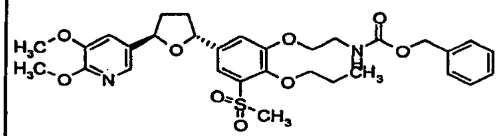
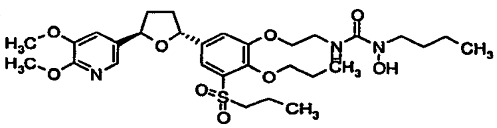
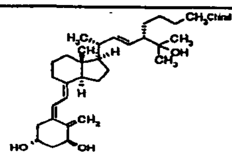
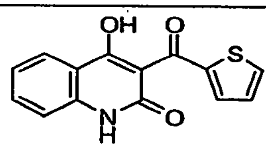
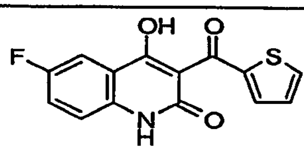
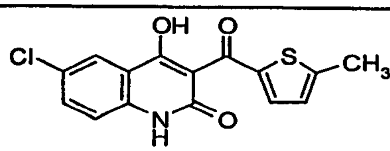
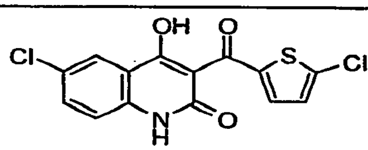
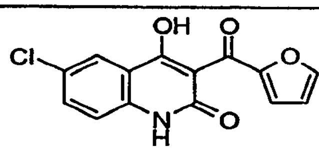
1306		Abbott GmbH		WO 9500493
1307		Abbott GmbH		WO 9500493
1308		Abbott GmbH		WO 9500493
1309		Abbott GmbH		WO 9500493
1310		Abbott GmbH		WO 9500493
1311		Abbott GmbH		WO 9500493
1312		Abbott GmbH		WO 9500493
1313		Abbott GmbH		WO 9500493
1314		Sanofi-Synthelabo		EP 644197
1315		Sanofi-Synthelabo		CA 2125021
1316		GlaxoSmithKline		WO 9504734

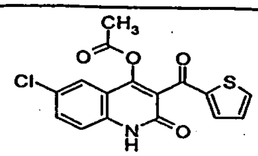
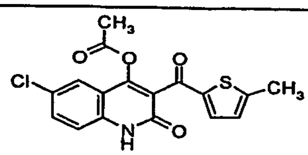
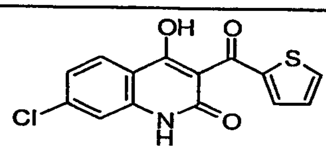
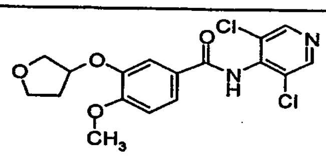
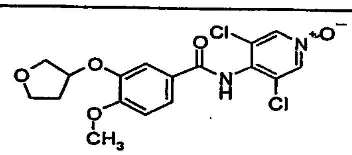
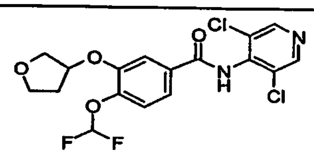
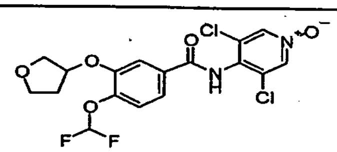
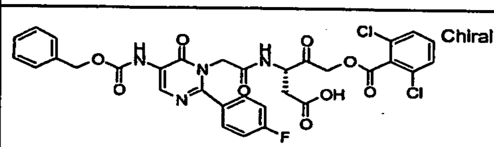
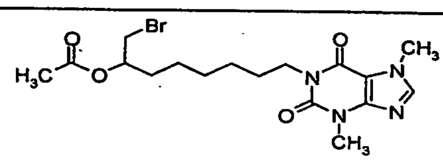
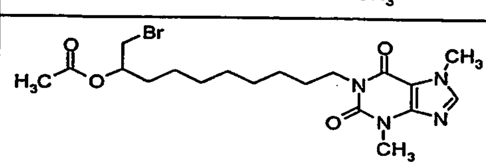
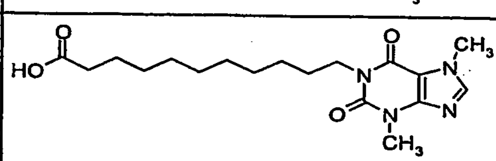
1317		GlaxoSmithKline		WO 9504734
1318		GlaxoSmithKline		WO 9504734
1319		GlaxoSmithKline		WO 9504734
1320		GlaxoSmithKline		WO 9504734
1321		GlaxoSmithKline		WO 9504734
1322		Japan Tobacco		JP 95002779
1323		Pharmacia	1) Gozzi, P. et al. J Pharmacol Exp Ther 1999, 291(1): 199.	JP 1995501330
1324	Chiral 	Sanofi-Synthelabo		EP 644197
1325	Chiral 	Sanofi-Synthelabo		EP 644197
1326	Chiral 	Sanofi-Synthelabo		EP 644197
1327	Chiral 	Sanofi-Synthelabo		CA 2125021

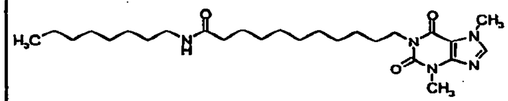
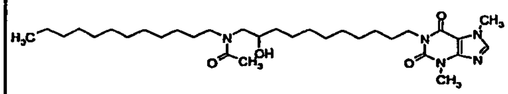
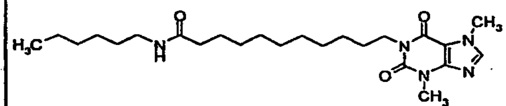
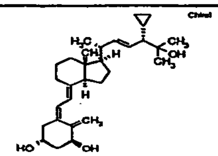
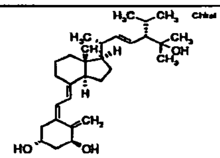
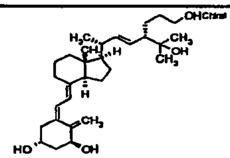
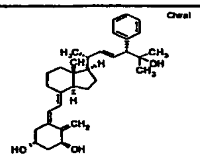
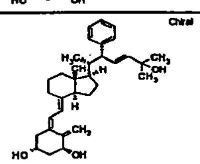
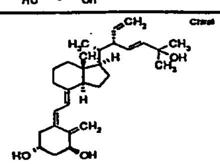
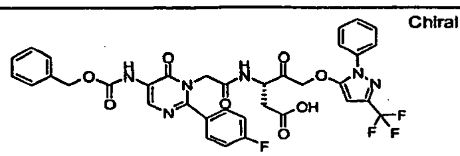
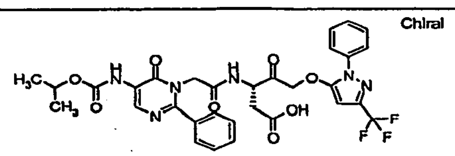
1328		Sanofi-Synthelabo		CA 2125021
1329		Sanofi-Synthelabo		CA 2125021
1330		Sanofi-Synthelabo		CA 2125021
1331		Sanofi-Synthelabo		CA 2125021
1332		Sanofi-Synthelabo		CA 2125021
1333		Sanofi-Synthelabo		CA 2125021
1334		Lilly		WO 9517382
1335		Millennium		WO 9518610
1336		Aventis Pharma		WO 9520578
1337		Cell Therapeutics		WO 9522546
1338		Lilly		WO 9517382

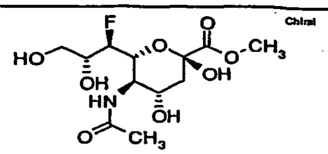
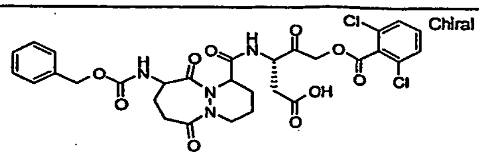
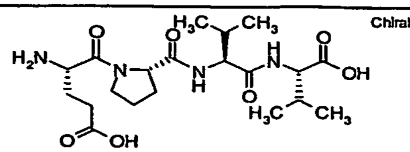
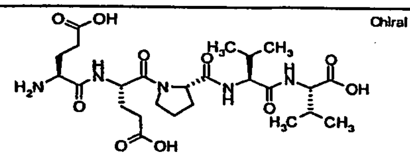
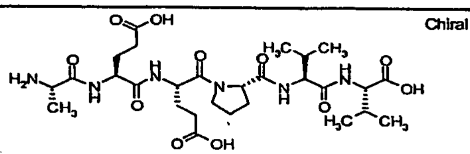
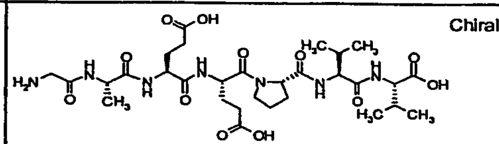
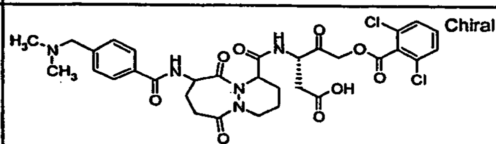
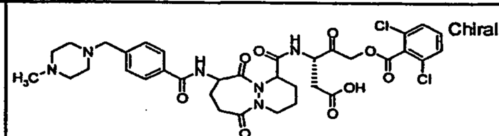
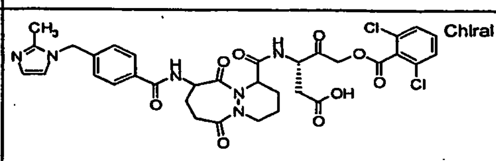
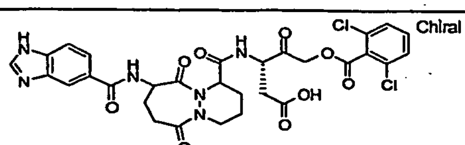
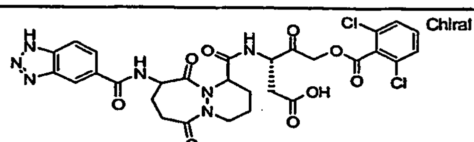
1339		Lilly		WO 9517382
1340		Lilly		WO 9517382
1341		Lilly		WO 9517382
1342		Lilly		WO 9517382
1343		Lilly		WO 9517382
1344		Lilly		WO 9517382
1345		Lilly		WO 9517382
1346		Lilly		WO 9517382
1347		Lilly		WO 9517382
1348		CytoMed		WO 9518610
1349		CytoMed		WO 9518610

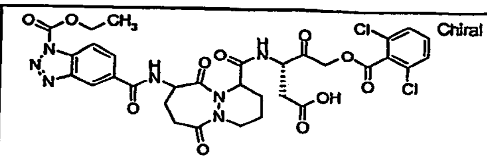
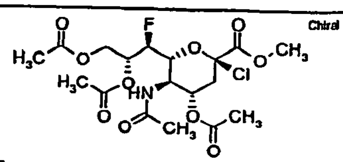
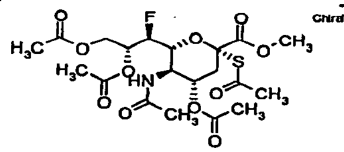
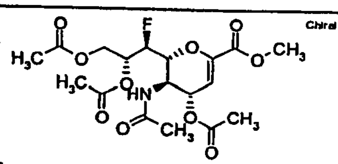
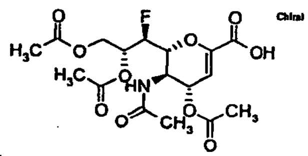
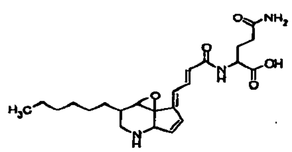
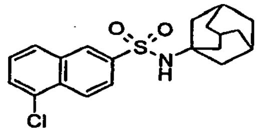
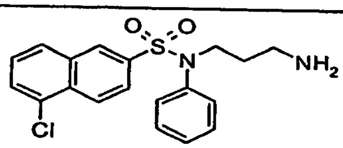
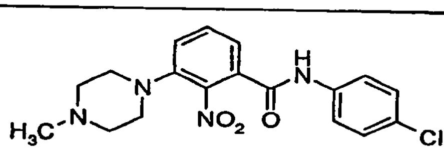
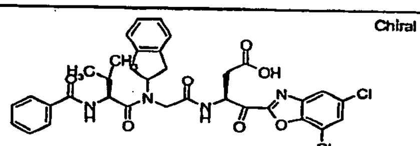
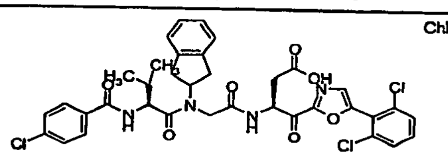
1350		CytoMed		WO 9518610
1351		CytoMed		WO 9518610
1352		CytoMed		WO 9518610
1353		CytoMed		WO 9518610
1354		CytoMed		WO 9518610
1355		CytoMed		WO 9518610
1356		CytoMed		WO 9518610
1357		CytoMed		WO 9518610
1358		CytoMed		WO 9518610
1359		CytoMed		WO 9518610
1360		CytoMed		WO 9518610

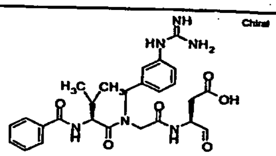
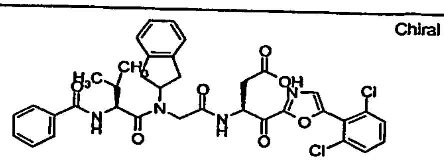
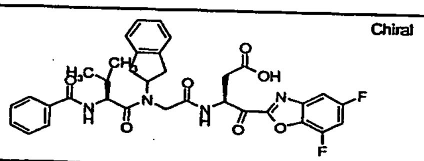
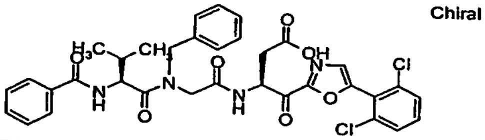
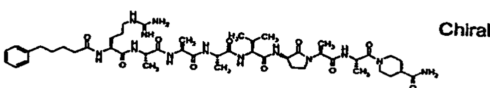
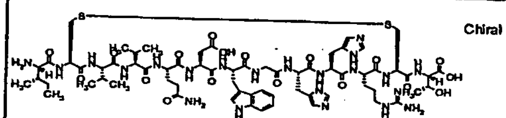
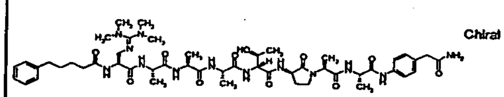
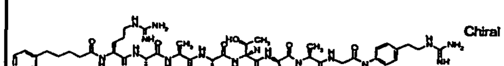
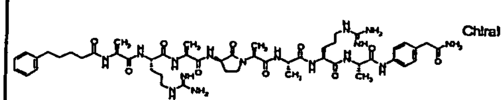
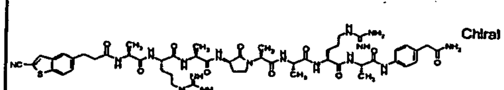
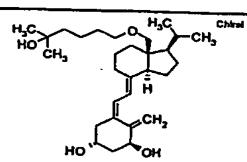
1361		CytoMed		WO 9518610
1362		CytoMed		WO 9518610
1363		CytoMed		WO 9518610
1364		CytoMed		WO 9518610
1365		CytoMed		WO 9518610
1366		Duphar		EP 664287
1367		Japan Tobacco		JP 95002779
1368		Japan Tobacco		JP 95002779
1369		Japan Tobacco		JP 95002779
1370		Japan Tobacco		JP 95002779
1371		Japan Tobacco		JP 95002779

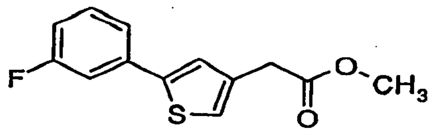
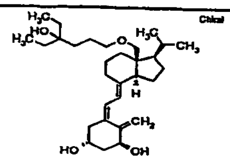
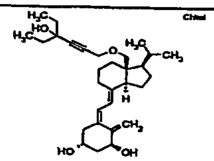
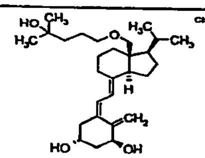
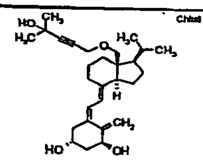
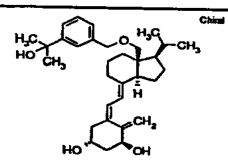
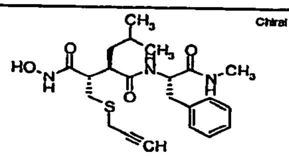
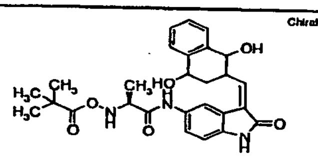
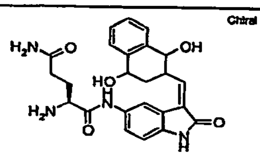
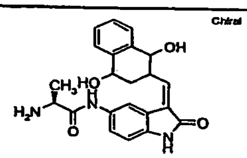
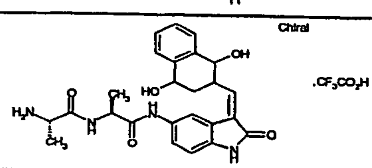
1372		Japan Tobacco		JP 95002779
1373		Japan Tobacco		JP 95002779
1374		Japan Tobacco		JP 95002779
1375		Aventis Pharma		WO 9520578
1376		Aventis Pharma		WO 9520578
1377		Aventis Pharma		WO 9520578
1378		Aventis Pharma		WO 9520578
1379		Sanofi-Synthelabo		WO 9526958
1380		Cell Therapeutics		WO 9522546
1381		Cell Therapeutics		WO 9522546
1382		Cell Therapeutics		WO 9522546

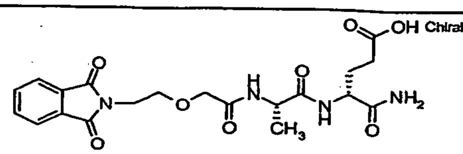
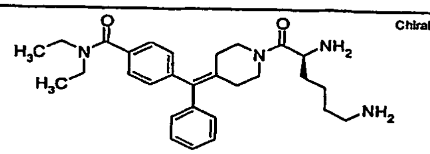
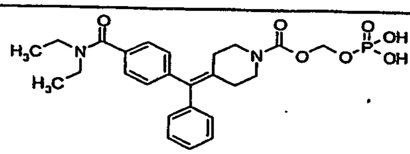
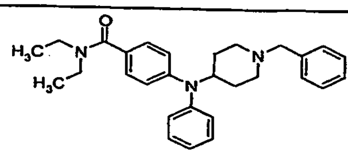
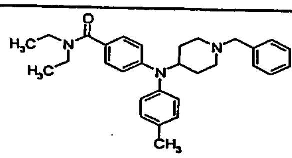
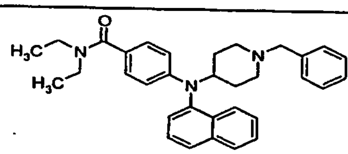
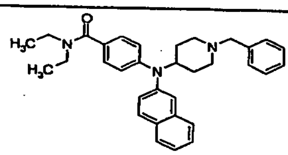
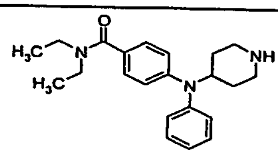
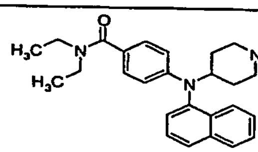
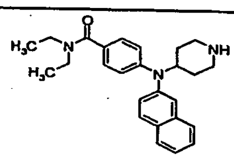
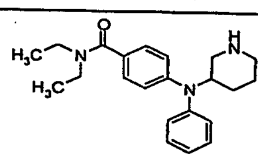
1383		Cell Therapeutics		WO 9522546
1384		Cell Therapeutics		WO 9522546
1385		Cell Therapeutics		WO 9522546
1386		Duphar		EP 664287
1387		Duphar		EP 664287
1388		Duphar		EP 664287
1389		Duphar		EP 664287
1390		Duphar		EP 664287
1391		Duphar		EP 664287
1392		Sanofi-Synthelabo		WO 9526958
1393		Sanofi-Synthelabo		WO 9526958

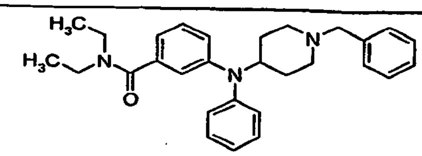
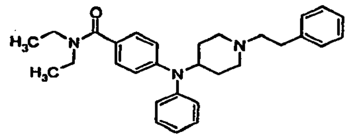
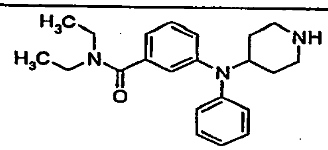
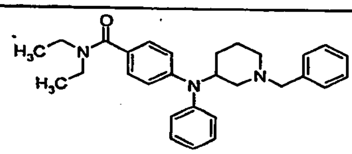
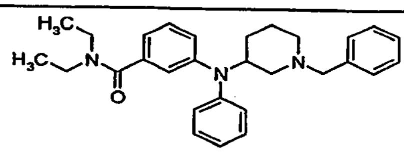
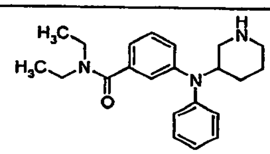
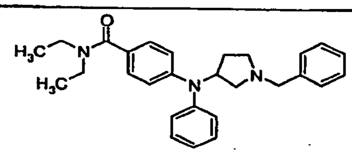
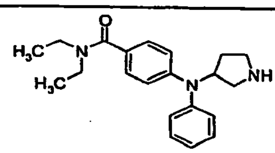
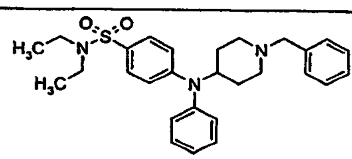
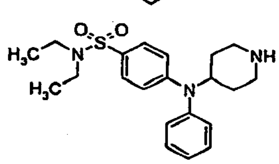
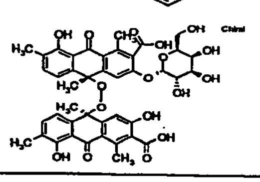
1394		Daikin		EP 711766
1395		Sanofi-Synthelabo		WO 9533751
1396		Ajinomoto		US 5464819
1397		Ajinomoto		US 5464819
1398		Ajinomoto		US 5464819
1399		Ajinomoto		US 5464819
1400		Sanofi-Synthelabo		WO 9533751
1401		Sanofi-Synthelabo		WO 9533751
1402		Sanofi-Synthelabo		WO 9533751
1403		Sanofi-Synthelabo		WO 9533751
1404		Sanofi-Synthelabo		WO 9533751

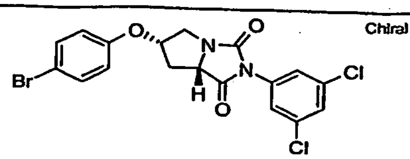
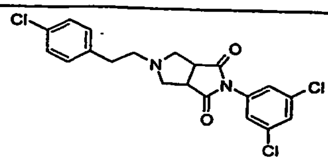
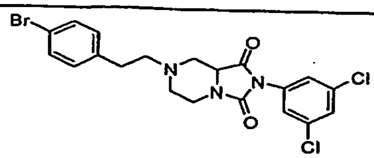
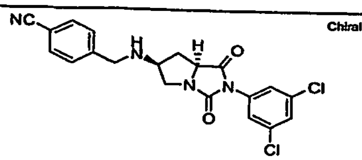
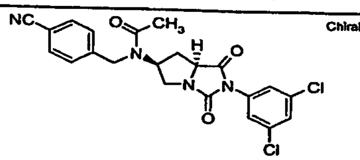
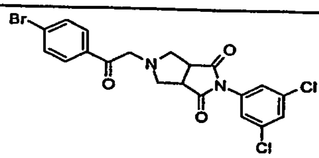
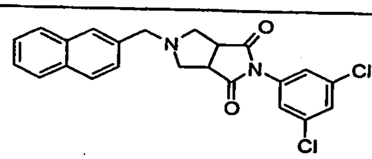
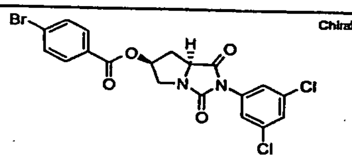
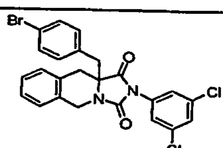
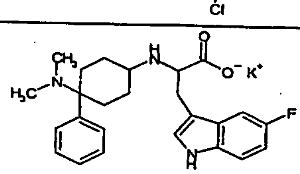
1405		Sanofi-Synthelabo		WO 9533751
1406		Daikin		EP 711766
1407		Daikin		EP 711766
1408		Daikin		EP 711766
1409		Daikin		EP 711766
1410		Microbial Chemistry Research Foundation		JP 96176157
1411		Tanabe		WO 9640641
1412		Tanabe		WO 9640641
1413		Daiichi Pharmaceutical	1) Kawagoe, K. et al. AFMC Int Med Chem Symp (Sept 3-8, Tokyo) 1995, Abst P13M183.	JP 97059236
1414		Vertex		WO 9722618
1415		Vertex		WO 9722618

1416		Vertex		WO 9722618
1417		Vertex		WO 9722618
1418		Vertex		WO 9722618
1419		Vertex		WO 9722618
1420		AstraZeneca		WO 9731023
1421		University of Pennsylvania		WO 9733603
1422		AstraZeneca		WO 9731023
1423		AstraZeneca		WO 9731023
1424		AstraZeneca		WO 9731023
1425		AstraZeneca		WO 9731023
1426		Leo		WO 9737972

1427		Taisho		JP 97194476
1428		Leo		WO 9737972
1429		Leo		WO 9737972
1430		Leo		WO 9737972
1431		Leo		WO 9737972
1432		Leo		WO 9737972
1433		GlaxoSmithKline		WO 9743250
1434		Pharmacia		WO 9745409
1435		Pharmacia		WO 9745409
1436		Pharmacia		WO 9745409
1437		Pharmacia		WO 9745409

1438		LEK		EP 477912
1439		AstraZeneca		WO 9828275
1440		AstraZeneca		WO 9828275
1441		AstraZeneca		WO 9828270
1442		AstraZeneca		WO 9828270
1443		AstraZeneca		WO 9828270
1444		AstraZeneca		WO 9828270
1445		AstraZeneca		WO 9828270
1446		AstraZeneca		WO 9828270
1447		AstraZeneca		WO 9828270
1448		AstraZeneca		WO 9828270

1449		AstraZeneca		WO 9828270
1450		AstraZeneca		WO 9828270
1451		AstraZeneca		WO 9828270
1452		AstraZeneca		WO 9828270
1453		AstraZeneca		WO 9828270
1454		AstraZeneca		WO 9828270
1455		AstraZeneca		WO 9828270
1456		AstraZeneca		WO 9828270
1457		AstraZeneca		WO 9828270
1458		AstraZeneca		WO 9828270
1459		Daiichi Pharmaceutical	Koiwa, T. et al. J Antibiot 1999, 52(2): 198.	

1460		Bristol-Myers Squibb		WO 0244181
1461		Bristol-Myers Squibb		WO 0244181
1462		Bristol-Myers Squibb		WO 0244181
1463		Bristol-Myers Squibb		WO 0244181
1464		Bristol-Myers Squibb		WO 0244181
1465		Bristol-Myers Squibb		WO 0244181
1466		Bristol-Myers Squibb		WO 0244181
1467		Bristol-Myers Squibb		WO 0244181
1468		Bristol-Myers Squibb		WO 0244181
1469		Gruenenthal		WO 0290317

BRIEF DESCRIPTION OF SEQUENCE LISTING

SEQ ID NO:	Description
1	Draft genome assembly from The Genome Science Center in British Colombia, Canada of sequence from TOR2 isolate. *TOR2_draft_genome_assembly_120403 Release 1*
2	CDC SARS-CoV strain sequence. Entire nucleotide sequence (Urbani strain)
3-20	Group-specific coronavirus gene products > Feline infectious peritonitis virus (FIPV) 3/4 = ORF 3b; 5/6 = ORF 3X; 7/8 = ORF 3A > Canine coronavirus 9/10 = ORF 7b; 11/12 = ORF 7a > Avian infectious bronchitis virus 13/14 = ORF 5b; 15/16 = ORF 5a; 17/18 = ORF 3a; 19/20 = ORF 3b
21-520	500 primers for left part
521-1020	500 primers for right part
1021-3520	Forward primers from Table 4
3521-6020	Reverse primers from Table 4
6021-6026	Figure 9 primers
6027-6033	Figure 11 primers
6034-6038	Five primers from *http://content.nejm.org/cgi/reprint/NEJMoa030781v2.pdf*
6039-6051	PEP1 to PEP13
6052	Extended PEP13
6053-6056	229E human coronavirus sequences
6057-6060	TGV sequences
6061-6064	PEDV sequences
6065-6068	Bovine coronavirus sequences
6069-6071	Murine hepatitis virus sequences
6072-6075	AIBV sequences
6076-6170	Primer sequences (forward)
6171-6265	Primer sequences (reverse)
6266-6304	Primer sequences (forward)
6305-6343	Primer sequences (reverse)
6344-6366	Primer sequences (forward)
6367-6392	Primer sequences (reverse)
6393-6440	Primer sequences (forward) F1-F48
6441-6487	Primer sequences (reverse) R1-R47
6488-6559	Primer sequences
6560-6568	Primer sequences
6569	The nsp2 proteinase (3CL-PRO) sequence in SARS coronavirus
6570-72	The nsp2 proteinases (3CLp) of avian IBV, MHV, and BCoV
6573	Consensus nsp2 proteinases sequence
6574-6577	IG sequences from Figure 18
6578	Expression construct of nSh in pCMVIII
6579	Expression construct of nS in pCMVIII
6580	Expression construct of nSh ΔTC in pCMVIII
6581	Expression construct of nS ΔTC in pCMVIII
6582	Expression construct of nS1h in pCMVIII
6583	Expression construct of nS1 in pCMVIII
6584-6585	Primers for cDNA amplification
6585-6587	Primers for RT-PCR
6588-6809	Component sequences of Figure 23 (≥4 amino acids)
6810-7179	Component sequences of Figure 24 (≥4 amino acids)
7180-7187	N-glycosylation sites within SEQ ID NO: 6039
7188-7189	Component sequences of Figure 25
7190	Fragment of SEQ ID NO: 7188
7191	Polynucleotide encoding SEQ ID NO: 7190
7192	Amino acids 879-1005 of SEQ ID NO: 6042
7193	Amino acids 879-980 of SEQ ID NO: 6042

7194	Amino acids 901-1005 of SEQ ID NO: 6042
7195	Amino acids 1144-1201 of SEQ ID NO: 6042
7196	Amino acids 1144-1196 of SEQ ID NO: 6042
7197-7199	Membrane fusion peptide regions
7200-7206	NadA-based polypeptides
7207-7223	N-glycosylation sites within SEQ ID NO: 6042
7224-7231	Slippage region
7232	Orf1ab polyprotein
7233-7244	Orf1ab polyproteins
7245-7247	X_2 sequences for SEQ ID NOS 7233-7244
7248-7253	Orf1ab polyproteins
7254	Zinc binding region 2 site
7255-7271	N-glycosylation sites in SEQ ID NOS: 6040-41,6043,6045-46,6050-51
7272-7291	Polypeptides and polynucleotides
7292-7293	Intergenic sequences
7294-7301	Nucleotides from 5' end of SARSV genome followed by intergenic sequence
7302-7306	NadA constructs
7307-7308	Fragments of SEQ ID NO: 6042
7309	NadA sequence
7310-7311	NadA leader sequences
7312-7315	Amino acid sequencess from NadA
7316-7324	PCR primers
7325-7330	Primers
7331	CCACC sequence
7332-7336	3' UTR forward primers
7337-7341	3' UTR reverse primers
7342-7352	3' UTR probes
7353-7362	5' UTR forward primers
7363-7373	5' UTR reverse primers
7374-7385	5' UTR probes
7386	Conserved octanucleotide
7387	Reverse complement of SEQ ID NO: 7293
7388	Intergenic sequence
7389	Poly T
7390	Stem-loop sequence
7391-7392	Poly-glycine linkers
7393	Poly-histidine tag
7394	Nucleocapsid epitope site
7395	Antisense primer
7396-7397	Probes
7398-7399	Antigenic fragments of SEQ ID NO: 6042
7400-7639	T-epitope analysis of SEQ ID NO: 6039
7640-7800	T-epitope analysis of SEQ ID NO: 6040
7801-8040	T-epitope analysis of SEQ ID NO: 6041
8041-8280	T-epitope analysis of SEQ ID NO: 6042
8281-8486	T-epitope analysis of SEQ ID NO: 6043
8487-8665	T-epitope analysis of SEQ ID NO: 6044
8666-8820	T-epitope analysis of SEQ ID NO: 6045
8821-9018	T-epitope analysis of SEQ ID NO: 6046
9019-9131	T-epitope analysis of SEQ ID NO: 6047
9132-9308	T-epitope analysis of SEQ ID NO: 6048
9309-9437	T-epitope analysis of SEQ ID NO: 6049
9438-9538	T-epitope analysis of SEQ ID NO: 6050
9539-9752	T-epitope analysis of SEQ ID NO: 6052
9753-9763	Primers for spike protein amplification, particularly fragments of spike
9764-9765	N-glycosylation sites within SEQ ID NO: 6039
9766-9779	Cleavage products for ORF1ab (Table 10)

9780-9782	Forward primer, reverse primer, probe
9783-9784	Lysine-rich region
9785-9798	Oligonucleotides used for *S.cerevisiae* expression
9799-9802	Sequences from Figures 65 & 66
9803-9882	Primers for *E.coli* cloning
9883-9885	BCV nucleotide sequences for Figures 3A, 3B, 3C
9886-9891	BCV amino acid sequences for Figures 4A, 4B, 4C, 4D, 4E, 4F
9892	BCV 5' UTR
9893	BCV 3' UTR
9894-9896	MHV nucleotide sequences for Figures 3A, 3B, 3C
9897-9902	MHV amino acid sequences for Figures 4A, 4B, 4C, 4D, 4E, 4F
9903-9904	AIBV nucleotide sequences for Figures 3A, 3B
9905-9909	AIBV amino acid sequences for Figures 4A, 4B, 4D, 4E, 4F
9910	AIBV 5' UTR
9911	AIBV 3' UTR
9912-9913	HOBMPRO, HOBHEGA nucleotide sequences for Figures 3B, 3C
9914-9918	Human CoV amino acid sequences for Figures 4A, 4B, 4C, 4E, 4F
9919	HCoV-OC43 5' UTR
9920	HCoV-OC43 3' UTR
9921-9923	pCMVKm2 vectors
9924-9926	Codon-optimised N, M and E sequences
9927	BNI-1
9928-9959	Constituent amino acid sequences ≥4aa inferred from SEQ ID NO: 9927
9960	ORF1ab variant
9961	ORF1a variant
9962	Spike variant
9963	Membrane variant
9964	Nucleocapsid variant
9965-9966	Short ORFs
9967	FRA complete genome

CLAIMS

1. An isolated polypeptide of the SARS virus.

2. The polypeptide of claim 1, wherein the polypeptide is a Spike (S) polypeptide, an Env (E) polypeptide, a Membrane (M) polypeptide, a hemagglutinin-esterase polypeptide (HE), a nucleocapsid (N) polypeptide, a ORF1a polypeptide, a ORF1ab polypeptide, a proteolytic fragment of a ORF1a polypeptide, or a proteolytic fragment of a ORF1ab polypeptide.

3. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO^s: 6039, 7232, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050 or 6052.

4. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence having >75% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.

5. The polypeptide of claim 1, wherein the polypeptide comprises a fragment of at least 10 consecutive amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.

6. A polypeptide comprising an amino acid sequence having >80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.

7. A polypeptide comprising an amino acid sequence that comprises a fragment of at least 10 consecutive amino acids of an amino acid sequence selected from the group consisting SEQ ID NO^S: 6042, 6043, 6044, 6045, 6046, 6047, 6048, 6049, 6050, 6052, 9766, 9767, 9768, 9769, 9770, 9771, 9772, 9773, 9774, 9775, 9776, 9777, 9778, 9779, 9997, 9998, 10149, 10316, 10338, 10339, 10340, 10341, 10342, 10532, 10533, 10571, 10572, 10573, 10574, 10575, 10576, 10577, 10578, 10579, 11552, 11561, 11562, 11618, 11619, 11620, 11627, 11630, 11633 & 11636.

8. A polypeptide comprising an amino acid sequence having >80% sequence identity to SEQ ID NO: 6042, and/or comprising an amino acid sequence that comprises a fragment of at least 10 consecutive amino acids of SEQ ID NO: 6042, wherein the polypeptide is in the form of a trimer.

9. Nucleic acid encoding the polypeptide of any one of claims 1 to 8.

10. Nucleic acid according to claim 9, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO^{S}: 7191, 7273, 7275, 7277, 7279, 7281, 7283, 7285, 7287, 7289, 7291, 7292, 7293, 9968, 10066, 10084, 10299, 10505, 11323, 11563, 11639 & 11640.

11. A polynucleotide comprising a nucleotide sequence having >80% sequence identity to the nucleic acid of claim 9 or claim 10.

12. A polynucleotide comprising a fragment of at least 10 consecutive nucleotides of the nucleic acid of claim 9 or claim 10.

13. Antibody that recognizes the polypeptide of any one of claim 1 to 8.

14. The antibody of claim 13, wherein said antibody recognizes the polypeptide comprising the amino acid sequence of SEQ ID NO: 6042 or a fragment thereof.

15. The antibody of claim 14, wherein said antibody recognizes the polypeptide comprising the amino acid sequence of SEQ ID NO: 6042 or a fragment thereof in trimeric form.

16. The antibody of claim 13, wherein the antibody is a monoclonal antibody,

17. The antibody of claim 13, wherein the antibody is a human antibody,

18. An immunoassay for detecting a SARS virus antigen in a sample, comprising the step of contacting the sample with the antibody of any one of claims 13 to 17.

19. An immunoassay for detecting an antibody against a SARS virus antigen in a sample, comprising the step of contacting the sample with the polypeptide of any one of claims 1 to 8.

20. A method of detecting an antibody against a SARS virus antigen in a sample comprising contacting said sample with the polypeptide of any one of claims 1 to 8, under conditions suitable for binding said polypeptide to said antibody, if present, and detecting the binding of said polypeptide to said antibody.

21. A method for detecting a SARS virus antigen in a sample comprising contacting said sample with the antibody of any one of claims 13 to 17, under conditions suitable for binding said antibody to said antigen, if present, and detecting the binding of said antibody to said antigen.

22. A vaccine for the treatment or prevention of severe acute respiratory syndrome (SARS), comprising an inactivated SARS virus, a killed SARS virus, an attenuated SARS virus, a split SARS virus preparation, or at least one purified SARS virus antigens.

23. The vaccine of claim 22, comprising a purified polypeptide according to any one of claims 1 to 8.

24. The vaccine of claim 22 or claim 23, wherein the antigen is a purified SARS virus antigen in the form of a VLP.

25. The vaccine of any one of claims 22 to 24, further comprising an adjuvant.

26. The vaccine of claim 25, wherein the adjuvant is an aluminium salt or is MF59.

27. The vaccine of any one of claims 22 to 26, comprising more than one SARS virus antigen.

28. The vaccine of claim 27, wherein the antigens are selected from S, E, N and M.

29. The vaccine of claim 22, comprising an inactivated SARS virus.

30. The vaccine of claim 29, wherein said virus is inactivated by chemical or physical means.

31. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of detergents, formaldehyde, formalin, β-propriolactone, and UV light.

32. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of methylene blue, psoralen and carboxyfullerene (C60).

33. The vaccine of claim 30, wherein said inactivation comprises treatment of the virus with an effective amount of one or more of the following agents selected from the group consisting of binary ethylamine, acetyl ethyleneimine and gamma irradiation.

34. The vaccine of claim 31, wherein said inactivation comprises treatment with β-propriolactone.

35. The vaccine of claim 34, wherein said β-propriolactone is used at a concentration of 0.01 to 0.5%.

36. The vaccine of claim 34, wherein said β-propriolactone is used at a concentration of 0.5 to 0.2%.

37. The vaccine of claim 34, wherein said β-propriolactone is used at a concentration of 0.025 to 0.1%.

38. A method of inactivating SARS virus comprising exposing the virus to an inactivation agent for 12 to 24 hours at refrigeration temperatures followed hydrolysis of any residual inactivating agent by elevating the temperature for three hours.

39. The method of claim 38, wherein the inactivation agent is β-propriolactone.

40. The method of claim 38, wherein the refrigeration temperature is between 0°C and 8°C.

41. The method of claim 38, wherein the elevated temperature is between 33°C and 41°C.

42. A method for making an inactivated SARS vaccine comprising:

a. innoculating a mammalian cell culture with SARS virus;

b. cultivating the infected cells;

c. harvesting SARS virus containing supernatant;

d. inactivating the SARS virus; and

e. purifying the inactivated SARS virus.

43. The method of claim 42, wherein said mammalian cell culture is derived from one or more of the cell types selected from the group consisting of fibroblast cells, endothelial cells, hepatocytes, keratinocytes, immune cells, mammary cells, smooth muscle cells, melanocyte cells, neural cells, prostate cells, renal cells, skeletal cells, liver cells, retinoblast cells and stromal cells.

44. The method of claim 42, wherein said mammalian cell culture is derived from a cell culture selected from the group consisting of human cells, non-human primate cells, HeLa cells, human diploid cells, fetal rhesus lung cells, human embryonic kidney cells, VERO cells, horse cells, cow cells, sheep cells, dog cells, cat cells or rodent cells.

45. The method of claim 42, wherein said mammalian cell culture is derived from VERO cells or fetal rhesus kidney cells.

46. The method of claim 42, wherein said mammalian cells are cultured in serum free media.

47. The method of claim 42, wherein said mammalian cells are cultured in protein free media.

48. The method of claim 42, wherein said inoculating step comprising absorbing the SARS virus onto the cell culture for 60 to 300 minutes.

49. The method of claim 42, wherein said inoculating step is conducted at 25°C to 40°C.

50. The method of claim 42, wherein said purification step comprises one or more of the treatments selected from the group consisting of gradient centrifugation, ultracentrifugation, continuous-flow ultracentrifugation, chromatography, polyethylene glycol precipitation, and ammonium sulfate precipitation.

51. The method of claim 42, wherein said purification step comprises one or more of the treatments selected from the group consisting of ultrafiltration and dialfiltration.

52. The method of claim 50, wherein said chromatography treatment includes one or more of the chromatography treatments selected from the group consisting of ion exchange chromatography, size exclusion chromatography, and liquid affinity chromatography.

53. The method of claim 52, wherein said chromatography treatment includes use of one more chromatographic resins selected from the group consisting of an an anionic resin and a cationic resin.

54. The method of claim 52, wherein the ion exchange chromatography treatment includes a first step using a strong anion exchange resin and a second step using a strong cation exchange resin.

55. The method of claim 50, wherein said gradient centrifugation purification step comprises density gradient centrifugation.

56. The method of claim 42, wherein said purification step comprises a first step of chromatography purification and a second step of gradient centrifugation.

57. The method of claim 56, wherein said first chromatography purification step comprises liquid affinity chromatography.

58. The method of claim 56, wherein said second gradient centrifugation step comprises density gradient centrifugation.

59. A single-stranded oligonucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

60. A single-stranded oligonucleotide comprising the complement of the oligonucleotide of claim 59.

61. The oligonucleotide of claim 59 or claim 60, comprising 10-30 nucleotides.

62. The oligonucleotide of claim 61, comprising the nucleotide sequence of SEQ ID NO: 7292, SEQ ID NO: 7293, the complement of SEQ ID NO: 7292 or the complement of SEQ ID NO: 7293.

63. A kit comprising primers for amplifying a template sequence contained within a SARS virus nucleic acid target, the kit comprising a first primer and a second primer, wherein the first primer comprises a sequence substantially complementary to a portion of said template sequence and the second primer comprises a sequence substantially complementary to a portion of the

complement of said template sequence, wherein the sequences within said primers which have substantial complementarity define the termini of the template sequence to be amplified.

64. The kit of claim 63, wherein the template sequence is contained within SEQ ID NO: 1 and/or SEQ ID NO: 2.

65. The kit of claim 63 or claim 64, wherein the first primer comprises a fragment of 8 or more nucleotides of SEQ ID NO: 1, and the second primer comprises a fragment of 8 or more nucleotides of the complement of SEQ ID NO: 1.

66. The kit of claim 63 or claim 64, wherein the first primer comprises a fragment of 8 or more nucleotides of SEQ ID NO: 2, and the second primer comprises a fragment of 8 or more nucleotides of the complement of SEQ ID NO: 2.

67. The kit of claim 63, wherein the first primer is an oligonucleotide according to any one of claims 59 to 62 and the second primer is an oligonucleotide according to any of claims 59 to 62.

68. The kit of any one of claims 63 to 67, further comprising a labeled probe that comprises either a fragment of 8 or more nucleotides of SEQ ID NO: 1 and/or SEQ ID NO: 2, or the complement of said fragment, which fragment is located within the template sequence.

69. The kit of any one of claims 63 to 68, wherein the first primer and/or the second primer comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

70. The kit of any one of claims 63 to 68, wherein the first primer and/or the second primer comprises the complement of a nucleotide sequence selected from the group consisting of SEQ ID NOS: 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322 and 11325-11551.

71. A method of detecting the presence of SARS virus in a sample comprising providing a sample suspected of containing a SARS virus nucleic acid target, amplifying a template sequence contained within said SARS virus nucleic acid target with the kit of any one of claims 63 to 70, and detecting the amplified template sequence, wherein the presence of the amplified template sequence indicates the presence of SARS virus in said sample.

72. The method of claim 71, wherein said amplifying is accomplished using polymerase chain reaction, transcription mediated amplification, reverse transcription PCR, ligase chain reaction, strand displacement amplification or nucleic acid sequence-based amplification.

73. A double-stranded RNA molecule with a length from about 10 to about 30 nucleotides which is able to inactivate the SARS coronavirus in a mammalian cell.

74. The double-stranded RNA of claim 73, wherein the sequence of one of the strands is at least 90% identical to a target sequence, wherein the target sequence is a fragment of SEQ ID NO: 1 and/or SEQ ID NO: 2.

75. The double-stranded RNA of claim 73 or claim 74, wherein the target sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 7292, 7293, 7294, 7295, 7296, 7297, 7298, 7299, 7300 and 7301.

76. The double-stranded RNA of any one of claims 73 to 75, comprising at least one modified nucleotide.

77. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a molecule of less than 1000 g/mol.

78. The method of claim 77, wherein the molecule has an aromatic region and greater than one heteroatom selected from O, S, or N.

79. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a compound selected from: a nucleoside analog, a peptoid, an oligopeptide, a polypeptide a protease inhibitor, a 3C-like protease inhibitor , a papain-like protease inhibitor, or an inhibitor of an RNA dependent RNA polymerase.

80. A method for treating a patient suffering from SARS, comprising: administering to the patient a steroidal anti-inflammatory drug in combination with at least one antiviral compound.

81. A method for treating a patient suffering from SARS, comprising: administering to the patient a therapeutically effective dose of a compound selected from: acyclovir, gancyclovir, vidarabidine, foscamet, cidofovir, amantidine, ribavirin, trifluorothymidine, zidovudine, didanosine, zalcitabine, an antiviral compound listed in Table 1; an antiviral compound listed in Table 2; or an interferon.

82. The method of claim 81, wherein the interferon is an interferon-α or an interferon-β.

83. The method of any one of claims 77 to 82, wherein the molecule or compound is delivered by inhalation.

84. A method of identifying a therapeutically active agent comprising the steps of: (a) contacting a therapeutically active agent with a cell infected with the SARS virus; (b) measuring attenuation of a SARS related enzyme.

85. A viral vector or particle for *in vivo* delivery of a nucleic acid of claim 9 or claim 10.

86. The viral vector of claim 85, wherein the vector is an adenovirus vector, a poxvirus vector or an alphavirus vector.

87. An alphavirus replicon particle comprising one or more SARS viral antigens.

88. The replicon particle of claim 87, wherein said SARS viral antigen is a spike protein.

89. The replicon particle of claim 87, wherein said particle comprises a replicon derived from Venezuelan Equine Encephalitis (VEE) and further comprises an envelope derived from Sindbus virus (SIN) or Semliki Forest Virus (SFV).

90. A vaccine comprising one or more SARS virus antigens and one or more respiratory virus antigens.

91. The vaccine of claim 90, wherein said respiratory virus antigens are selected from the group consisting of influenza virus, human rhinovirus (HRV), parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, and rhinovirus.

92. The vaccine of claim 91, wherein said respiratory virus antigen is from influenza virus.

93. The vaccine of claim 90, wherein said respiratory virus antigen is from a coronavirus other than the SARS virus.

94. A polypeptide comprising an immunogenic, surface exposed fragment of the amino acid sequence SEQ ID NO: 6042.

95. The polypeptide of claim 94, wherein said fragment does not include the last 50 amino acids of the C-terminus of SEQ ID NO: 6042.

96. The polypeptide of claim 94, wherein said fragment does not include a transdomain region of SEQ ID NO: 6042.

97. The polypeptide of claim 94, wherein said fragment does not include a C-terminus cytoplasmic domain of SEQ ID NO: 6042.

98. The polypeptide of claim 94, wherein said fragment does not include a N-terminus signal sequence.

99. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066.

100. The polynucleotide of claim 99, wherein the polynucleotide comprising a nucleic acid sequence having > 80% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066.

101. An isolated polynucleotide comprising a fragment of at least 15 consecutive nucleic acids of a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 9968 and 10066 and wherein said fragment does not consist entirely of SEQ ID NO: 10033.

102. An isolated polypeptide comprising an amino acid sequence encoded by any one of claims 99 – 101.

103. The polypeptide of claim 102, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 9969 – 10032, 10067, and 10015.

104. The polypeptide of claim 103, wherein the amino acid sequence is selected from the group consisting of SEQ ID NOS: 9997, 9998 and 10015.

105. An expression construct for recombinant expression of a SARS virus spike protein wherein said construct comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 6578 – 6583.

106. A mammalian cell line stably expressing a SARS viral antigen.

107. The cell line of claim 106, wherein said cell line is a Chinese Hamster Ovary (CHO) cell.

108. The cell line of claim 106, wherein the SARS viral antigen is a spike protein or fragment thereof.

109. The cell line of claim 106, wherein the spike protein is truncated to remove the transmembrane sequence.

110. A method of identifying a therapeutically active agent comprising the steps of: (a) contacting a therapeutically active agent with a buffer comprising SARS enzyme; and (b) measuring attenuation of the SARS enzyme.

111. The method of claim 110 wherein the SARS enzyme is a SARS protease.

112. The method of claim 111 wherein the buffer further comprises a peptide with a SARS protease cleave site.

113. The method of claim 110 wherein the measurement is made by the measurement of fluorescence.

114. A vaccine of one of claims 22 to 37, and 90 to 93 further comprising an adjuvant.

115. The vaccine of claim 114 wherein the adjuvant is a SMIP.

116. The vaccine of claim 115 wherein the SMIP compound is selected from the group consisting of an acylpiperazine, a tryptanthrin, an indoledione, a tetrahydroisoquinoline, a benzocyclodione, an amino azavinyl compound, a thiosemicarbazone, a lactam, an aminobenzimidazole quinolinone, a hydropthalamide, a benzophenone, an isoxazole, a sterol, a quinazolinone, a pyrole, an anthraquinone, a quinoxaline, a triazine, an benzazole, and a pyrazolopyrimidine, or a pharmaceutically acceptable salt, ester, or prodrug thereof.

117. A method of vaccinating a subject comprising administering a vaccine of one of claims 22 to 37, and 90 to 93.

118. The method of claim 117 further comprising administering a SMIP.

119. A method for treating a patient of one of claims 77 to 82 further comprising administering at least one SMIP compound.

120. A method for treating a patient of one of claims 77 to 82 further comprising administering at least one SMIS compound.

FIGURE 1

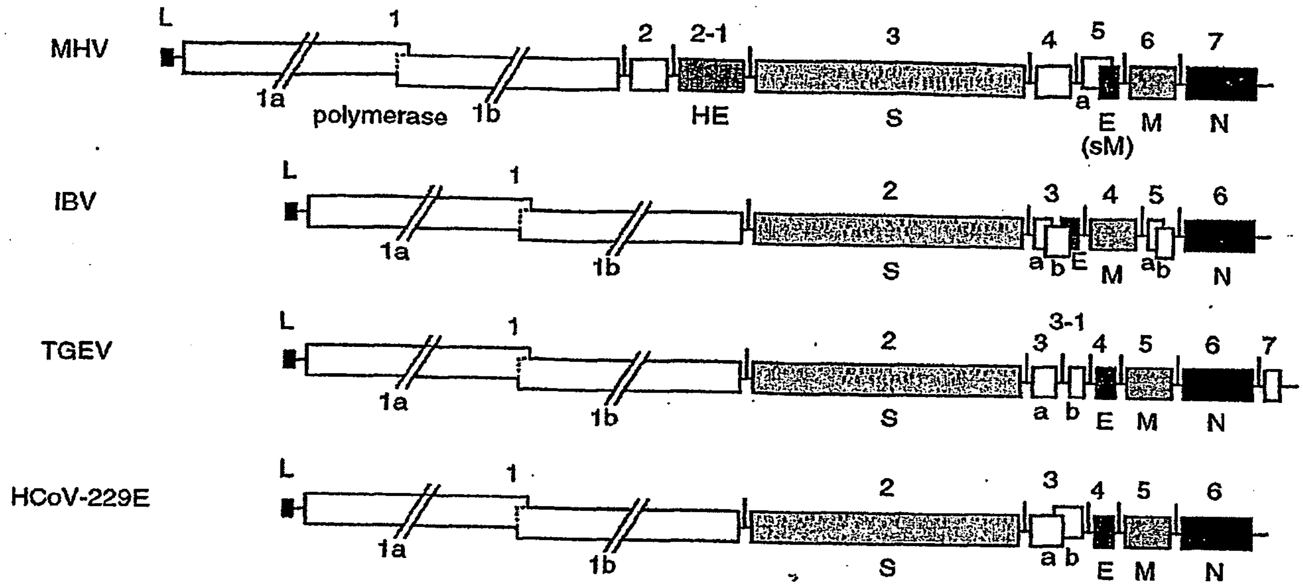


FIGURE 2

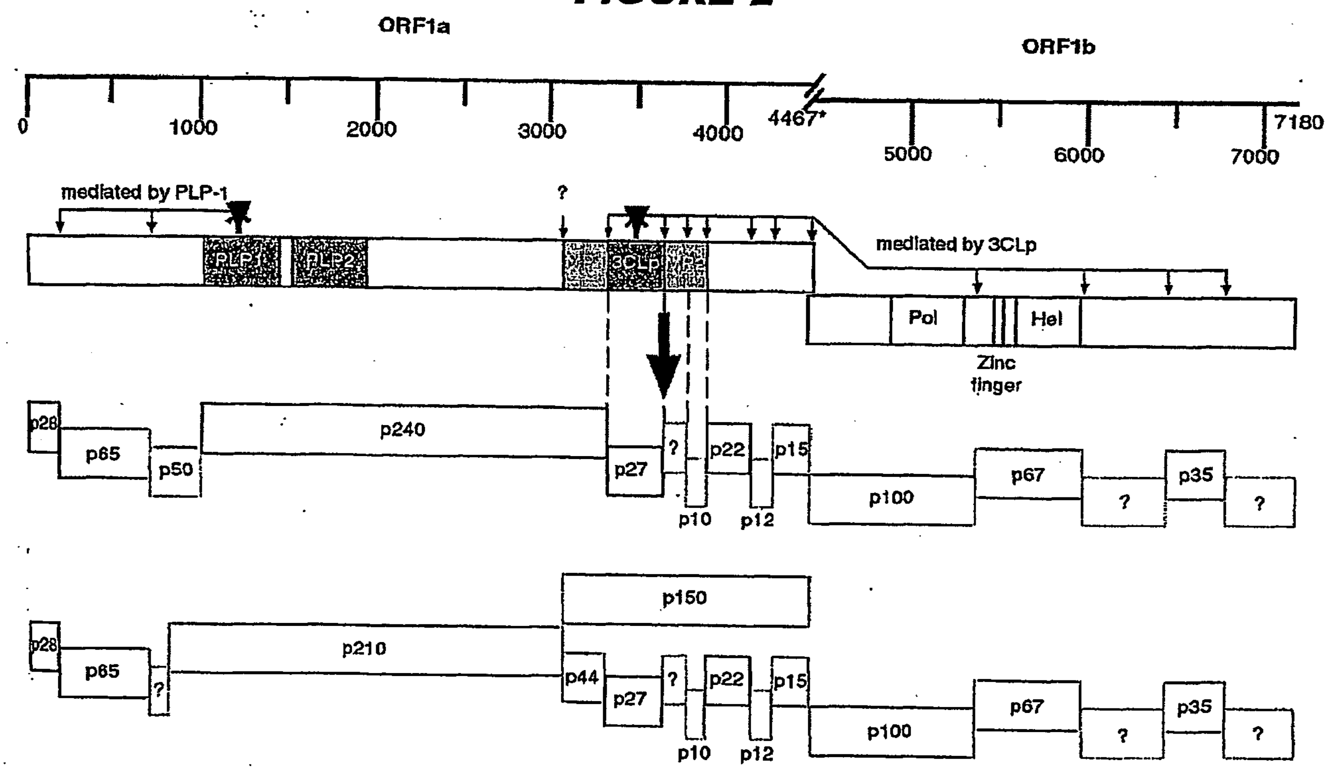


FIGURE 3

FIGURE 3A

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                                                                          Section 1
                                  (1) 1        10        20        30          43
                          BCV N   (1) ATGTCTTTTACTCCTGGT-AAGCAAT--CCAGTAGTAGAGCGT
                          MHV N   (1) ATGTCTTTTGTTCGTGGCAAGAAAATGCCGGTGGCAGAAGCT
Avian infectious  bronchitis N    (1) -------------------------------------------
                      Consensus   (1) ATGTCTTTT  TCCTGG  AAG AA   CC GT G AGA    T
                                                                          Section 2
                                 (44) 44   50        60        70              86
                          BCV N  (41) CCTTTGCAAATCGTTCTGGTAATGGCATCCTTAAG--------
                          MHV N  (44) CCTCTGTAAACCGCGCTGGTAATGGAATCCTCAAGAAGACCAC
Avian infectious  bronchitis N    (1) -------------------------------------------
                      Consensus  (44) CCT TG AAA CG   CTGGTAATGG ATCCT AAG
                                                                          Section 3
                                 (87) 87          100       110               129
                          BCV N  (76) -TGGGCCGATCAGTCCGACCAATCTAGAAATGTTGAAACCAGG
                          MHV N  (87) TTGGGGTGACCAAAACCGAGCGTGGACCAAATAATCAAAATAGA
Avian infectious  bronchitis N    (1) -----------ATGGCGAGCGGTAAAGCAGCTGGAAAGTCAGA
                      Consensus  (87)  TGGGC GA CA   CCGAGCG T AAGAAAT  TCAAA CAGA
                                                                          Section 4
                                (130) 130       140       150       160       172
                          BCV N (118) GGTAGAAGAGGTCAAGCCAAGGAAACTGCTACTTCTCAGCTAC
                          MHV N (130) GGCAGAAGGAATCAGCCAAAGCAGACTGCAACTACTCAAC--C
Avian infectious  bronchitis N  (33) CTGCCCCGCGCCAATC---ATCAAACTAGGAGGACCAAAA--C
                      Consensus (130) GGCAGAAG GCTCA CC AAGCAAACTGC ACTACTCAAC  C
                                                                          Section 5
                                (173) 173    180       190       200          215
                          BCV N (161) CATCAGGAGGGAATGTTGTACGCTACTAT-TCTTGGTTCTCTG
                          MHV N (171) CAACTCC-GGGAGTGTGGTTCCCCATTAC-TCCTGGTTTTCTG
Avian infectious  bronchitis N  (71) CACCAAA-GCTAGGGTCATCTGGAAATGCATCTTGGTTTCAAG
                      Consensus (173) CA CA    GGGAGTGT GT CCC  A TAC TCTTGGTTTTCTG
                                                                          Section 6
                                (216) 216           230       240             258
                          BCV N (203) GAATTA---CTCAGTTTCAAAAAGGAAAGGACTTTGAATTTGC
                          MHV N (212) GCATTA---CCCAGTTCCAAAAGGGAAAGGAGTTTCAGTTTGC
Avian infectious  bronchitis N (113) CCATAAAGGCCAAGAAACTAAATGCACCTGCACCTAAGTTTG-
                      Consensus (216) GCATTA    CCCAGTT CAAAA GGAAAGGAGTTT AGTTTGC
                                                                          Section 7
                                (259) 259       270       280       290       301
                          BCV N (243) AGAGGGACAAGGTGTGCCTATTGCACCAGGAGTCCCAGCTACT
                          MHV N (252) AGAAGGACAAGGAGTGCCTATTGCCAATGGAATCCCCGCTTCA
Avian infectious  bronchitis N (155) --AAGGTAGTGGTGTTCCTGATAATGAAAATCTTAAAAATAGC
                      Consensus (259) AGAAGGACAAGGTGTGCCTATTGC  AAGGA TCCCAGCTAC
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FIGURE 3A (contd.)

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                                                                        Section 8
                                   (302) 302       310        320        330           344
                            BCV N  (286) GAAGCTAAGGGGTACTGGTACAGACACAACAGACGTTCTTTTA
                            MHV N  (295) GAGCAAAAGGGATATTGGTATAGACACAACCGCCGTTCTTTTA
Avian infectious    bronchitis N   (196) CAGCAGCACGGGTACTGGAGGCGCCAAGCCAG------GTTTA
                        Consensus  (302) GAGCA AAGGGGTACTGGTA AGACACAACAG CGTTCTTTTA
                                                                        Section 9
                                   (345) 345  350        360        370           387
                            BCV N  (329) AAACAGCCGATGGCA-ACCAGCGTCAACTGCTGCCACGATGGT
                            MHV N  (338) AAACACCTGATGGGC-AGCAGAAGCAATTACTGCCCAGATGGT
Avian infectious    bronchitis N   (233) AGCCAGGTAAAGGCGGAAGAAAACCAGTCCCTGATGCG-TGGT
                        Consensus  (345) AAACAGCTGATGGC  A CAGAA CAATT CTGCC CGATGGT
                                                                        Section 10
                                   (388) 388         400        410        420        430
                            BCV N  (371) ATTTTTACTATCTTGGAACAGGACCGCATGCCAAAGACCAGTA
                            MHV N  (380) ATTTTTACTATCTTGGCAGAGGGCGCCATGCTGGAGCCAGTTA
Avian infectious    bronchitis N   (275) ATTTCTATTACACTGGAACAGGACCAGCCGCTGACCTGAATTG
                        Consensus  (388) ATTTTTACTATCTTGGAACAGGACC CATGCTGAAG CAATTA
                                                                        Section 11
                                   (431) 431      440        450        460           473
                            BCV N  (414) TGGCACCGATATTGACGGAGTCTTCTGGGTCGCTAGTAACCAG
                            MHV N  (423) TGGAGACAGCATTGAAGGTGTCTTCTGGGTTGCAAACAGCCAA
Avian infectious    bronchitis N   (318) GGGTGATTCTCAAGATGGTATAGTGTGGGTTGCTGCTAAGGGT
                        Consensus  (431) TGG GAC  TATTGA GGTGTCTTCTGGGTTGCTA TAACCA
                                                                        Section 12
                                   (474) 474   480        490        500            516
                            BCV N  (457) GCTGATGTCAATACCCCGGCTGACATTCTCGATCGGGACCCAA
                            MHV N  (466) GCGGACACCAATACCCGCTCTGATATTGTCGAAAGGGACCCAA
Avian infectious    bronchitis N   (361) GCTGATGTGAAATCTAGATCCAACCAGGGTACTAGAGATCCTG
                        Consensus  (474) GCTGATGTCAATACCCG TCTGACATTGTCGATAGGGACCCAA
                                                                        Section 13
                                   (517) 517          530        540           559
                            BCV N  (500) GTAGCGATGAGGCTATTCCGACTA-GGTTTCGGCCTGGCACGG
                            MHV N  (509) GCAGTCATGAGGCTATTCCTACTA-GGTTTGCGGCCCGGCACGG
Avian infectious    bronchitis N   (404) ATAAGTTTGAT-CAATACCCACTACGGTTTTCAGATGG-----
                        Consensus  (517) GTAG   ATGAGGCTATTCC ACTA GGTTT CGCCTGGCACGG
                                                                        Section 14
                                   (560) 560       570        580        590           602
                            BCV N  (542) TACTCCCTCAGGGTTACTATATTGAAGGCTCAGGAAGGTCTGC
                            MHV N  (551) TATTGCCTCAGGGCTTTTATGTTGAAGGCTCTGGAAGGTCTGC
Avian infectious    bronchitis N   (441) -AGGACCTGATGGTAATT-----------TCCGTTGGGACTTC
                        Consensus  (560) TA T CCTCAGGGTTATTAT TTGAAGGCTC GGAAGGTCTGC
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FIGURE 3A (contd.)

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                                                                         Section 15
                                (603) 603      610                 620                 630              645
                         BCV N  (585) TCCTAATTCCAGATCTACTTCACGCGCA---TCCAGTAGAGCC
                         MHV N  (594) ACCTGCTAGCCGATCTGGTTCGCGGTCACAATCCCGTGG-GCC
Avian infectious bronchitis N  (472) AT-TCCTCTGAGTCGTGGTAGGAGTGGAAGATCAACCGCAGCA
                     Consensus  (603) ACCT CT  CAGATCTGGTTCGCG GCA  ATCCAGTGGAGCC
                                                                         Section 16
                                (646) 646                     660            670                   688
                         BCV N  (625) TCTAGTGCAGGATCGCGTAGTAGAGCCAATTCTGGCAACAGAA
                         MHV N  (636) AAATA------ATCGCG--CTAGAAGCAGTTCCAACCAGCGCC
Avian infectious bronchitis N  (514) TCATCAGCGGCATCTAG---TAGAGTACCATGGCGTGAGGGTT
                     Consensus  (646) TCAT  GC G ATCGCG   TAGAG CA TTCC GC AG G
                                                                         Section 17
                                (689) 689              700             710              720          731
                         BCV N  (668) CCCCTACCTCTGGTGTAACACCTGATATGGCTGATCAAATTGC
                         MHV N  (671) AGCCTGGCTCTACTGTAAAACCTGATATGGCCCAAGAAATTGC
Avian infectious bronchitis N  (554) CACGTGGTCGTAGGAGTGGAGCTGA---AGATGATCTGATTGC
                     Consensus  (689) C CCTGCCTCTAGTGTAA ACCTGATATGGCTGATCAAATTGC
                                                                         Section 18
                                (732) 732        740               750               760           774
                         BCV N  (711) TAGTCTTGTTCTGCCAAAACTTGGCAAGGATGCCACTAAGCCA
                         MHV N  (714) TGCTCTTGTTTTGGCTAAGCTCGGTAAAGATGCCGGCCAGCCC
Avian infectious bronchitis N  (594) CCGTGCAGCAAAGATTATTCAGGACCAGCAG---AGGAAGGGT
                     Consensus  (732) T GTCTTGTT TGGCTAA CT GGCAAGGATGCCAG AAGCC
                                                                         Section 19
                                (775) 775  780            790               800                  817
                         BCV N  (754) CAGCAAGTAACTAAGCAGACTGCCAAAGAAATCAGACA--GAA
                         MHV N  (757) AAGCAAGTAACGAAGCAAAGTGCCAAAGAAGTCAGGCA--GAA
Avian infectious bronchitis N  (634) ACGCGCATTACTAAGCAAAAGGCAGAAGAGATGGCTCATCGCC
                     Consensus  (775) AAGCAAGTAACTAAGCAAA TGCCAAAGAAATCAG CA  GAA
                                                                         Section 20
                                (818) 818                830              840              850       860
                         BCV N  (795) AATTTTGAATAAGCCCCGCCAGAAGAGGAGCCCCAATAAACAA
                         MHV N  (798) AATTTTAAACAAGCCTCGCCAAAAGAGGACTCCAAACAAGCAG
Avian infectious bronchitis N  (677) GATTCTGTAAGCGTACGGTGCCACCAGGTTATAGAGTAGATCA
                     Consensus  (818) AATTTTGAA AAGCCCCGCCA AAGAGGA  CC AATAAACAA
                                                                         Section 21
                                (861) 861         870              880              890           903
                         BCV N  (838) TGCACTGTTCAGCAGTGTTTTGGGAAGAGAGGCCCCAATCAGA
                         MHV N  (841) TGCCCAGTGCAGCAGTGTTTTGGAAAGAGAGGCCCCAATCAGA
Avian infectious bronchitis N  (720) AGTTTTTGGCCCTCGTACTAAAGGTAAAGAGGGT--------A
                     Consensus  (861) TGC CTGTGCAGCAGTGTTTTGGGAAGAGAGGCCCCAATCAGA
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FIGURE 3A (contd.)

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                                                                              Section 22
                                 (904) 904     910          920          930              946
                        BCV N    (881) ATTTTGGTGGTGGAGAAATGTTAAAACTTGGAACTAGTGACCC
                        MHV N    (884) ATTTTGGAGGCTCTGAAATGTTAAAACTTGGAACTAGTGATCC
Avian infectious bronchitis N    (755) ATTTTGGTGATGACAAGATGAATGAGGAAGGTATTAAGGATGG
                    Consensus    (904) ATTTTGGTGGTG  GAAATGTTAAAACTTGGAACTAGTGATCC
                                                                              Section 23
                                 (947) 947            960        970                      989
                        BCV N    (924) ACAGTTCCCCATTCTTGCAGAAC-TCGCACCCACAGCTGGTGC
                        MHV N    (927) ACAGTTCCCCATTCTTGCAGAGT-TGGCTCCAACAGTTGGTGC
Avian infectious bronchitis N    (798) GC-GTGTTACAGCAATGCTCAACCTTACACCAAGCCCACATGC
                    Consensus    (947) ACAGTTCCCCATTCTTGCAGAAC T GCACCAACAGCTGGTGC
                                                                              Section 24
                                 (990) 990        1000       1010       1020            1032
                        BCV N    (966) GTTTTTCTTTGGATCAAGATTAGAGTTGGCCAAAGTGCAGAAT
                        MHV N    (969) CTTCTTCTTTGGATCTAAATTAGAATTGGTCAAAA---AGAAT
Avian infectious bronchitis N    (840) TTGTCTTTTTGGAAGTAGAGT-GAC---GGCCAAG--------
                    Consensus    (990)  TTTTTCTTTGGATCTAGATTAGA TTGGCCAAAG   AGAAT
                                                                              Section 25
                                (1033) 1033   1040        1050       1060              1075
                        BCV N   (1009) TTGTCTGGGAATCTTGACGAGCCCCAGAAGGATGTTTATGAAT
                        MHV N   (1009) T---CTGGTGGTGCTGATGAACCCACCAAAGATGTGTATGAGC
Avian infectious bronchitis N    (871) ----CT--TCAACCAGATGGGCTTC---ACCTTAGATTTGAAT
                    Consensus   (1033) T   CTGGT ATCCTGATGAGCCCC  AA GATGT TATGAAT
                                                                              Section 26
                                (1076) 1076             1090       1100                1118
                        BCV N   (1052) TGCGCTATAATGGTGCAATTAGATTTGACAGTACACTTTCAGG
                        MHV N   (1049) TGCAATATTCAGGTGCAGTTAGATTTGATAGTACTCTACCTGG
Avian infectious bronchitis N    (905) TT-ACTACTGTGGTGCC--TAGAGATGACCCGCAGTTTGATAA
                    Consensus   (1076) TGCACTATT TGGTGCA TTAGATTTGACAGTAC CTT CTGG
                                                                              Section 27
                                (1119) 1119          1130       1140       1150        1161
                        BCV N   (1095) TTTTGAGACCATA----ATGAAGGTGTTGAATGAGAATTTGAA
                        MHV N   (1092) TTTTGAGACTATC----ATGAAAGTGTTGAATGAGAATTTGAA
Avian infectious bronchitis N    (945) TTATGTAAAAATTTGTGATGAGTGTGTTGATGGTGTAG--GAA
                    Consensus   (1119) TTTTGAGAC AT      ATGAA GTGTTGAATGAGAATTTGAA
                                                                              Section 28
                                (1162) 1162    1170       1180       1190              1204
                        BCV N   (1134) TGCATATCAACAACAAGATGGTAT-GATGAATATGA-GTCCAA
                        MHV N   (1131) TGCCTACCAGAAGGATGGTGGTGCAGATGTGG-TGA-GCCCAA
Avian infectious bronchitis N    (986) CACGTCCAAAGGACGAAGTTGTAA-GACCAAAGTCACGCCCAA
                    Consensus   (1162) TGC TACCAA AACAAGGTGGTA  GATGAA  TGA GCCCAA
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FIGURE 3A (contd.)

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                                                                   Section 29
                          (1205) 1205 1210      1220      1230          1247
                     BCV N (1175) AACCACAGCGTCA---GCGTGGTCAG----AAGAATGGACAAG
                     MHV N (1172) AGCGCCAAAGAAAAGGGCGTAGACAGGCTCAGGAAAAGA-AAG
Avian infectious bronchitis N (1028) ATTCAAGACCTGC--TACAAGAACAAG-TTCTCCAGCGCCAAG
                 Consensus (1205) A CCACAACGT A   GCGTGGACAGG T A GAA  GACAAG
                                                                   Section 30
                          (1248) 1248        1260      1270      1280     1290
                     BCV N (1211) GAGAAAATGATAATATAAGTGTTGCAGCGCCTAAAAGCCGTGT
                     MHV N (1214) ATGAAGTAGATAATGTAAGCGTTGCAAAGCCCAAAAGCTCTGT
Avian infectious bronchitis N (1068) ACAACAGCGTCAAAAGAAGGAGAAGAAGTCAAAGAAGGAGGAT
                 Consensus (1248) A GAAA  GATAATATAAG GTTGCAA GCC AAAAGC GTGT
                                                                   Section 31
                          (1291) 1291     1300      1310      1320        1333
                     BCV N (1254) GCAGCAAAATAAGAGTAGAGAGTTGACTGCAGA---GGACATC
                     MHV N (1257) GCAGCGAAATGTAAGTAGAGAATTAACGCCAGA---GGATAGA
Avian infectious bronchitis N (1111) GATGAAGTAGATAAG----GCATTGACCTGAGATGAGGAGAGG
                 Consensus (1291) GCAGCAAAATATAAGTAGAGAATTGACC CAGA   GGA AG
                                                                   Section 32
                          (1334) 1334  1340      1350      1360          1376
                     BCV N (1294) AGCCTTCT---TAAGAAGATGGATGA---------GCCCTATA
                     MHV N (1297) AGTCTGTTGGCTCAGATCCTTGATGATGGCGTAGTGCCAGATG
Avian infectious bronchitis N (1150) AAGAATGCACAGCTGGAATTTGATGATGAACCCAAGGTGATTA
                 Consensus (1334) AGCCTT T   TCAGAA  TTGATGATG     GCC  ATA
                                                                   Section 33
                          (1377) 1377         1390      1400          1414
                     BCV N (1325) CT---GAAGACACCTCAGAAATATAA------------
                     MHV N (1340) GGTTAGAAGATGACTCTAATGTGTAA------------
Avian infectious bronchitis N (1193) ACTGGGGGGATTCAGCACTTGGAGAGAATGAGTTGTAA
                 Consensus (1377)  T  GAAGAT CCTCA ATGTATAA
```

BCV N SEQ ID NO: 9883

MHV N SEQ ID NO: 9894

Avian infectious bronchitis N SEQ ID NO: 9903

FIGURE 3B

```
                                                                Section 1
                                    (1) 1       10        20        30        41
                          HOBMPRO   (1) GATGTGGATGACGTTTAGGTAATCCAAACATTATGAGTAGT
                            BCV M   (1) --------------------------------ATGAGTAGT
                            MHV M   (1) --------------------------------ATGACTAGT
Avian infectious brochitis virus M  (1) -----------------------------------------
                        Consensus   (1)                                 ATGAGTAGT
                                                                Section 2
                                   (42) 42      50        60        70        82
                          HOBMPRO  (42) AAAACTA---CTCCAGCACCAGTTTATATCTGGAGTGCTGA
                            BCV M  (10) GTAACTA---CACCAGCACCAGTTTACACCTGGACTGCTGA
                            MHV M  (10) ACCACTCAGGCTCCACAGCCTGTTTATCAGTGGACGGCTGA
Avian infectious brochitis virus M  (1) --ATGTC---CAACGCGGCAAATTGCACTCTTGACTG-TGA
                        Consensus  (42) A AACTC   CTCCAGCGCCAGTTTATCTCTGGACTGCTGA
                                                                Section 3
                                   (83) 83      90        100       110       123
                          HOBMPRO  (80) TGAAGCTATTAAATTCCTAAAGGAATGGAATTTTTCTTTGG
                            BCV M  (48) TGAAGCTATTAAATTCGTAAAGGAATGGAACTTTTCTTTGG
                            MHV M  (51) TGAGGCAATTCGATTCCTTAAGGAATGGAATTTCTCTCTCG
Avian infectious brochitis virus M (36) ACAGTCAGTTGAGCTTTTTAAAGAATATAATTTATTTATAA
                        Consensus  (83) TGAGGCTATTAAATTCCTTAAGGAATGGAATTTTTCTTTGG
                                                                Section 4
                                  (124) 124     130       140       150       164
                         HOBMPRO  (121) GTATTATACTACTTTTTATTACAATCATATTGCAATTTGGA
                            BCV M  (89) GTATTATACTACTTTTTATTACAATCATATTGCAATTTGGA
                            MHV M  (92) GCATTATACTACTTTTTGTTACTATGATACTACAGTTCGGT
Avian infectious brochitis virus M (77) CCGCATTCCTATTGTTTCTTACTATACTACTTCAGTATGGA
                       Consensus  (124) GTATTATACTACTTTTTATTACTATCATATTGCAGTTTGGA
                                                                Section 5
                                  (165) 165     170       180       190       205
                         HOBMPRO  (162) TATACAAGTCGCAGTATGTTTGTTTATGTTATTAAGATGAT
                           BCV M  (130) TATACAAGTCGCAGTATGTTTGTTTATGTTATTAAGATGAT
                           MHV M  (133) TACACGAGCCGTAGCATGTTTGTTTATGTTGTGAAAATGAT
Avian infectious brochitis virus M (118) TATGCAACAAGGAGTCGGTTTATTTACATAATGAAAATGAT
                       Consensus  (165) TATACAAGTCGCAGTATGTTTGTTTATGTTATTAAGATGAT
                                                                Section 6
                                  (206) 206           220       230           246
                         HOBMPRO  (203) TATTTTGTGGCTTATGTGGCCCCTTACTATAATCTTAACTA
                           BCV M  (171) CATTTTGTGGCTTATGTGGCCCCTTACTATCATCTTAACTA
                           MHV M  (174) ACTTTTGTGGCTTATGTGGCCACTAACTATTGTTTTGTGTA
Avian infectious brochitis virus M (159) AGTGTTATGGTGCTTTTGGCCCCTTAACATTGCAGTAGGTG
                       Consensus  (206) AATTTTGTGGCTTATGTGGCCCCTTACTATTGTCTTAAGTA
```

FIGURE 3B (contd.)

```
                                                      Section 7
                                      (247) 247          260       270             287
                            HOBMPRO (244) TTTTCAATTGCGTATACGCATTGAATAATGTGTATCTTGGC
                              BCV M (212) TTTTCAATTGCGTGTATGCGTTGAATAATGTGTATCTTGGC
                              MHV M (215) TTTTTAACTGCGTCTATGCGCTAAATAATGTGTATCTTGGA
Avian infectious brochitis virus M (200) TAATTTCATGTATATATCCACCAAATACAGGAGGTCTTGTC
                          Consensus (247) TTTTTAATTGCGTATATGCGTTGAATAATGTGTATCTTGGC
                                                      Section 8
                                      (288) 288         300       310             328
                            HOBMPRO (285) CTTTCTATAGTTTTTACCATAGTGGCCATTATTATGTGGAT
                              BCV M (253) TTTTCTATAGTTTTCACTATAGTGGCCATTATCATGTGGAT
                              MHV M (256) TTTTCTATAGTGTTTACTATAGTGTCCATTATAATGTGGAT
Avian infectious brochitis virus M (241) GCAGCGAAAATACTTACAGTGGTTGCGTGTCTGTCTTTTGA
                          Consensus (288) TTTTCTATAGTTTTTACTATAGTGGCCATTAT ATGTGGAT
                                                      Section 9
                                      (329) 329         340       350             369
                            HOBMPRO (326) TGTGTATTTTGTGAATAGTATCAGGTTGTTTATTAGAACTG
                              BCV M (294) TGTGTATTTTGTGAATAGTATCAGGTTGTTTATTAGAACTG
                              MHV M (297) TATGTATTTTGTTAATAGCATCAGGTTGTTTATCAGGACTG
Avian infectious brochitis virus M (282) AGGATATTGGATTCAGAGTATCAGACTCTTTAAGCGGGGTG
                          Consensus (329) TGTGTATTTTGTTAATAGTATCAGGTTGTTTATTAGGACTG
                                                      Section 10
                                      (370) 370        380       390       400      410
                            HOBMPRO (367) GAAGTTTTTGGAGTTTCAACCCAGAAACAAACAACTTGATG
                              BCV M (335) GAAGTTGGTGGAGTTTCAACCCAGAAACAAACAACTTGATG
                              MHV M (338) GCAGCTGGTGGAGCTTCAACCCCGAAACAAACAACCTAATG
Avian infectious brochitis virus M (323) GGCAATGGTGGGCATTTAACCCTGAGTCTAA-----TGCCG
                          Consensus (370) GAAGTTGGTGGAGTTTCAACCCAGAAACAAACAACTTGATG
                                                      Section 11
                                      (411) 411       420       430       440       451
                            HOBMPRO (408) TGTATAGATATGAAAGGAACAATGTATGTTAGGCCGATAAT
                              BCV M (376) TGTATAGATATGAAGGGAAGGATGTATGTTAGGCCGATAAT
                              MHV M (379) TGTATAGATATGAAAGGTACTGTGTATGTTAGACCCATTAT
Avian infectious brochitis virus M (359) T----AGGT-TCAATACTCCTATCTA---ATGGTCAACAAT
                          Consensus (411) TGTATAGATATGAAAGGTACTATGTATGTTAGGCCGATAAT
                                                      Section 12
                                      (452) 452      460       470       480        492
                            HOBMPRO (449) TGAGGACTATCATACTCTGACGGTCACAATAATACGCGGCC
                              BCV M (417) TGAGGACTACCATACCCTTACGGTCACAATAATACGTGGTC
                              MHV M (420) AGAGGATTACCATACACTAACAGCCACTATCATTCGTGGTC
Avian infectious brochitis virus M (392) GTAATTTTGCTATAGA--GAGTGTGCCAAT-------GGTG
                          Consensus (452) TGAGGATTACCATACACTGACGGTCACAATAATACGTGGTC
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FIGURE 3B (contd.)

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                                                                     Section 13
                              (493) 493      500        510        520         533
                       HOBMPRO (490) ATGTTTACATTCAAGGTATAAAACTAGGTACTGGCTATTCT
                         BCV M (458) ATCTTTACATGCAAGGTATAAAACTAGGTACTGGCTATTCT
                         MHV M (461) ACCTCTATATGCAAGGTGTTAAGCTAGGCACTGGCTTCTCT
Avian infectious brochitis virus M (424) CTTTCT-----CCAATTATAAAGAATGGAGTTCTTTATTGT
                     Consensus (493) ATCTTTACATGCAAGGTATAAAGCTAGGTACTGGCTATTCT

                                                                     Section 14
                              (534) 534    540         550        560          574
                       HOBMPRO (531) TGGGCAGATTTGCCAGCTTATATGACTGTTGCTAAGGTTAC
                         BCV M (499) TTGTCAGATTTGCCAGCTTATGTGACTGTTGCTAAGGTCTC
                         MHV M (502) TTGTCTGATTTGCCTGCTTATGTTACAGTTGCTAAGGTGTC
Avian infectious brochitis virus M (460) GAGGGTCAGTGGCTTGC-----TAAATGTGAACCAGACCAC
                     Consensus (534) TTGTCTGATTTGCCTGCTTATGTGACTGTTGCTAAGGTCTC

                                                                     Section 15
                              (575) 575  580          590        600          615
                       HOBMPRO (572) ACACCTGTGCACATATAAGCGTGGTTTTCTTGACAGGATAA
                         BCV M (540) ACACCTGCTCACGTATAAGCGTGGTTTTCTTGACAAGATAG
                         MHV M (543) TCACCTTTGCACTTATAAGCGGCGCATTCTTAGACAAGGTAG
Avian infectious brochitis virus M (496) TTGCCTAAAGACATATTTG------TTTGCCCACCAGATAG
                     Consensus (575) TCACCTGTGCACATATAAGCGTGGTTTTCTTGACAAGATAG

                                                                     Section 16
                              (616) 616             630        640          656
                       HOBMPRO (613) GCGATACTAGTGGTTTTCCTGTTTATGTTAAGTCCAAAGTC
                         BCV M (581) GCGATACTAGTGGTTTTGCTGTTTATGTTAAGTCCAAAGTC
                         MHV M (584) ACGGTGTTAGCGGTTTTGCTGTTTATGTGAAGTCCAAGGTC
Avian infectious brochitis virus M (531) ACG-TAATA-----TCTACCGTATG-GTGCAG---AAATAC
                     Consensus (616) GCGATACTAGTGGTTTTGCTGTTTATGTTAAGTCCAAAGTC

                                                                     Section 17
                              (657) 657             670        680          697
                       HOBMPRO (654) GGTAATTACCGACTGCCATCAACCCAAAAGGGTTCTGGCAT
                         BCV M (622) GGTAATTACCGACTGCCATCAACCCAAAAGGGTTCTGGCAT
                         MHV M (625) GGAAATTACCGACTGCCCTCAAATAAACCGAGT---GGCAT
Avian infectious brochitis virus M (562) ACTGGTGACC-AAAGCGGAAATAAGAAAAGGTTTGCTACA-
                     Consensus (657) GGTAATTACCGACTGCCATCAACCCAAAAGGGTTCTGGCAT

                                                                     Section 18
                              (698) 698            710        720           738
                       HOBMPRO (695) GGACACCGCATTGTTGAGAAATAATATCTAAATTTTAAGGA
                         BCV M (663) GGACACCGCATTGTTGAGAAATAATATCTAA----------
                         MHV M (663) GGACACCGCATTGTTGAGA------ATCTAA----------
Avian infectious brochitis virus M (601) -----------------------------------------
                     Consensus (698) GGACACCGCATTGTTGAGAAATAATATCTAA

                                                                     Section 19
                              (739)
                       HOBMPRO (736) TG   SEQ ID NO: 9912
                         BCV M (694) --   SEQ ID NO: 9884
                         MHV M (688) --   SEQ ID NO: 9895
Avian infectious brochitis virus M (601) --   SEQ ID NO: 9904
                     Consensus (739)
```

FIGURE 3C

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                                                              Section 1
            (1) 1        10        20        30        40           53
HOBHEGA     (1) CTAAACTCAGTGAAAATGTTTTTGCTTCCTAGATTTATTCTAGTTAGCTGCAT
BCV HE      (1) CTAAACTCAGTGAAAATGTTTTTGCTTGTTAGATTTGTTCTAGTTAGCTGCAT
MHV HE      (1) -----------------------------------------------------
Consensus   (1) CTAAACTCAGTGAAAATGTTTTTGCTTC TAGATTT TTCTAGTTAGCTGCAT
                                                              Section 2
           (54) 54     60        70        80        90          106
HOBHEGA    (54) AATTGGTAGCTTAGGTTTTTACAACCCTCCTACCAATGTTGTTTCGCATGTAA
BCV HE     (54) AATTGGTAGCCTAGGTTTTGACAATCCTCCTACCAATGTTGTTTCGCATTTAA
MHV HE      (1) -----------------------------------------------------
Consensus  (54) AATTGGTAGC TAGGTTTT ACAA CCTCCTACCAATGTTGTTTCGCAT TAA
                                                              Section 3
          (107) 107          120       130       140               159
HOBHEGA   (107) ATGGAGATTGGTTTTTATTTGGTGACAGTCGTTCAGATTGTAATCATATTGTT
BCV HE    (107) ATGGAGATTGGTTTTTATTTGGTGACAGTCGTTCAGATTGTAATCATGTTGTT
MHV HE      (1) -----------------------------------------------------
Consensus (107) ATGGAGATTGGTTTTTATTTGGTGACAGTCGTTCAGATTGTAATCAT TTGTT
                                                              Section 4
          (160) 160       170       180       190       200         212
HOBHEGA   (160) AATATCAACCCCCATAATTATTCTTATATGGACCTTAATCCTGTTCTGTGTGA
BCV HE    (160) ACTACCAACCCCCGTAATTATTCTTATATGGACCTTAATCCTGCCTTGTGTGG
MHV HE      (1) -----------------------------------------------------
Consensus (160) A TA CAACCCCC TAATTATTCTTATATGGACCTTAATCCTG   TGTGTG
                                                              Section 5
          (213) 213    220       230       240       250            265
HOBHEGA   (213) TTCTGGTAAAATATCATCTAAAGCTGGCAACTCCATTTTTAGGAGTTTTCACT
BCV HE    (213) TTCTGGTAAAATATCATCTAAAGCTGGCAACTCCATTTTTAGGAGTTTTCACT
MHV HE      (1) -----------------------------------------------------
Consensus (213) TTCTGGTAAAATATCATCTAAAGCTGGCAACTCCATTTTTAGGAGTTTTCACT
                                                              Section 6
          (266) 266           280       290       300               318
HOBHEGA   (266) TTACCGATTTTTATAATTACACAGGCGAAGGTCAACAAATTATTTTTTATGAG
BCV HE    (266) TTACCGATTTTTATAATTACACAGGCGAAGGTCAACAAATTATTTTCTATGAG
MHV HE      (1) -----------------------------------------------------
Consensus (266) TTACCGATTTTTATAATTACACAGGCGAAGGTCAACAAATTATTTT TATGAG
                                                              Section 7
          (319) 319        330       340       350       360        371
HOBHEGA   (319) GGTGTTAATTTTACGCCTTATCATGCCTTTAAATGCAACCGTTCTGGTAGTAA
BCV HE    (319) GGTGTTAATTTTACGCCTTATCATGCCTTTAAATGCACCACTTCTGGTAGTAA
MHV HE      (1) -----------------------------------------------------
Consensus (319) GGTGTTAATTTTACGCCTTATCATGCCTTTAAATGCA C  TTCTGGTAGTAA
```

FIGURE 3C (contd.)

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                                                                         Section 8
          (372) 372      380            390            400            410           424
HOBHEGA   (372) TGATATTTGGATGCAGAATAAAGGCTTGTTTTATACTCAGGTTTATAAGAATA
BCV HE    (372) TGATATTTGGATGCAGAATAAAGGCTTGTTTTACACTCAGGTTTATAAGAATA
MHV HE      (1) -----------ATGGGCAATAAAGCTCGATTTTATGCCGAGTGTATGAGAAGA
Consensus (372) TGATATTTGGATGCAGAATAAAGGCTTGTTTTATACTCAGGTTTATAAGAATA

                                                                         Section 9
          (425) 425  430            440            450            460            477
HOBHEGA   (425) TGGCTGTGTATCGCAGCCTTACTTTTGTTAATGTACCATATGTTTATAATGGC
BCV HE    (425) TGGCTGTGTATCGCAGCCTTACTTTTGTTAATGTACCATATGTTTATAATGGC
MHV HE     (44) TGGCCCAATATAGGAGCCTATCGTTTGTTAATGTGTCTTATGCCTATGGAGGT
Consensus (425) TGGCTGTGTATCGCAGCCTTACTTTTGTTAATGTACCATATGTTTATAATGGC

                                                                         Section 10
          (478) 478         490            500           510           520        530
HOBHEGA   (478) TCTGCACAATCTACAGCTCTTTGTAAATCTGGTAGTTTAGTCCTTAATAACCC
BCV HE    (478) TCTGCACAATCTACAGCCCTTTGTAAATCTGGTAGTTTAGTTCTTAATAACCC
MHV HE     (97) AATGCAAAGCCCGCCTCCATTTGCAAAGACAATACTTTAACACTCAATAACCC
Consensus (478) TCTGCACAATCTACAGCCCTTTGTAAATCTGGTAGTTTAGT CTTAATAACCC

                                                                         Section 11
          (531) 531      540            550            560           570          583
HOBHEGA   (531) TGCATATATAGCTCCTCAAGCTAACTCTGGGGATTATTATTATAAGGTTGAAG
BCV HE    (531) TGCATATATAGCTCGTGAAGCTAATTTTGGGGATTATTATTATAAGGTTGAAG
MHV HE    (150) CACCTTCATATCGAAGGAGTCTAATTATGTTGATTATTACTATGAGAGTGAGG
Consensus (531) TGCATATATAGCTC TGAAGCTAATT TGGGGATTATTATTATAAGGTTGAAG

                                                                         Section 12
          (584) 584   590            600            610            620            636
HOBHEGA   (584) CTGATTTTTATTTGTCAGGTTGTGACGAGTATATCGTACCACTTTGTATTTTT
BCV HE    (584) CTGATTTTTATTTGTCAGGTTGTGACGAGTATATCGTACCACTTTGTATTTTC
MHV HE    (203) CTAATTTCACACTAGAAGGTTGTGATGAATTTATAGTACCGCTCTGTGGTTTT
Consensus (584) CTGATTTTTATTTGTCAGGTTGTGACGAGTATATCGTACCACTTTGTATTTTT

                                                                         Section 13
          (637) 637            650            660           670                  689
HOBHEGA   (637) AACGGCAAGTTTTTG----------TCGAATACA------AAGTATTATGATGA
BCV HE    (637) AACGGCAAGTTTTTG----------TCGAATACA------AAGTATTATGATGA
MHV HE    (256) AATGGCCATTCCAAGGGCAGCTCTTCGGATGCTGCCAATAAATATTATACTGA
Consensus (637) AACGGCAAGTTTTTG          TCGAATACA      AAGTATTATGATGA

                                                                         Section 14
          (690) 690        700            710            720            730         742
HOBHEGA   (675) TAGTCAATATTATTTTAATAAAGACACTGGTGTTATTTATGGTCTCAATTCTA
BCV HE    (675) TAGTCAATATTATTTTAATAAAGACACTGGTGTTATTTATGGTCTCAATTCTA
MHV HE    (309) CTCTCAGAGTTACTATAATATGGATATTGGTGTCTTATATGGGTTCAATTCGA
Consensus (690) TAGTCAATATTATTTTAATAAAGACACTGGTGTTATTTATGGTCTCAATTCTA
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FIGURE 3C (contd.)

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                                                                    Section 15
              (743) 743        750         760         770         780        795
    HOBHEGA   (728) C---------AGAAACCATTACCA------CTGGTTTTGATCTTAATTGTTAT
     BCV HE   (728) C---------TGAAAGCATTACCA------CTGGTTTTGACTTTAATTGTCAT
     MHV HE   (362) CCTTGGATGTTGGCAACACTGCTAAGGATCCGGGTCTTGATCTCACTTGCAGG
  Consensus   (743) C         TGAAACCATTACCA      CTGGTTTTGATCTTAATTGT AT
                                                                    Section 16
              (796) 796               810         820         830           848
    HOBHEGA   (766) TATTTAGTTTTACCCTCTGGTAATTATTTAGCCATTTCAAATGAGCTATTGTT
     BCV HE   (766) TATTTAGTTCTACGCTCTGGTAATTATTTAGCCATTTCAAATGAGCTATTGTT
     MHV HE   (415) TATCTTGCATTGACTCGTGGTAATTATAAGGGTGTGTCCTTAGAATATTTGTT
  Consensus   (796) TATTTAGTTTTACCCTCTGGTAATTATTTAGCCATTTCAAATGAGCTATTGTT
                                                                    Section 17
              (849) 849        860         870         880         890        901
    HOBHEGA   (819) AACTGTTCCTACGAAAGCAATCTGTCTTAATAAGCGTAAGGATTTTACGCCTG
     BCV HE   (819) AACTGTTCCTACTAAAGCAATCTGTCTTAATAAGCGTAAGGATTTTACGCCTG
     MHV HE   (468) AAGCTTACCCTGAAAGGCTATTTGCCTCCATAAGACAAAGCGCTTTATGCGTG
  Consensus   (849) AACTGTTCCTAC AAAGCAATCTGTCTTAATAAGCGTAAGGATTTTACGCCTG
                                                                    Section 18
              (902) 902        910         920         930         940        954
    HOBHEGA   (872) TACAGGTTGTTGATTCGCGGTGGAACAATGCCAGGCAGTCTGATAACATGACG
     BCV HE   (872) TACAGGTTGTTGATTCGCGGTGGAACAATGCCAGGCAGTCTGATAACATGACG
     MHV HE   (521) TGCAGGTAGTTGACTCAAGGTGGAGTAGCATGCGCCAGTCAGACAATATGACC
  Consensus   (902) TACAGGTTGTTGATTCGCGGTGGAACAATGCCAGGCAGTCTGATAACATGACG
                                                                    Section 19
              (955) 955   960         970         980         990            1007
    HOBHEGA   (925) GCGGTTGCTTGTCAAGCTCCGTACTGTTATTTTCGTAATTCTAGTACCAACTA
     BCV HE   (925) GCAGTTGCTTGTCAACCCCCGTACTGTTATTTTCGTAATTCTACTACCAATTA
     MHV HE   (574) GCTGCAGCCTGTCAGCTGCCATATTGTTTCTTTCGCAACACATCTGCGAATTA
  Consensus   (955) GC GTTGCTTGTCAACC CCGTACTGTTATTTTCGTAATTCTACTACCAATTA
                                                                    Section 20
             (1008) 1008            1020        1030        1040       1050   1060
    HOBHEGA   (978) TGTTGCTGTTT---ATGATATTAATCATGGAGATGCTGGTTTTACTAGCATAC
     BCV HE   (978) TGTTGGTGTTT---ATGATATCAATCATGGGGATGCTGGTTTTACTAGCATAC
     MHV HE   (627) TAGTGGTGGCACACATGATGCGCACCATGGTGATTTTCATTTCAGGCAGTTAT
  Consensus  (1008) TGTTGGTGTTT   ATGATAT AATCATGG GATGCTGGTTTTACTAGCATAC
                                                                    Section 21
             (1061) 1061      1070        1080        1090        1100       1113
    HOBHEGA  (1028) TTAGTGGTTTGTTATATAATTCACCTTGTTTTTCGCAGCAAGGCGTTTTTAGG
     BCV HE  (1028) TCAGTGGTTTGTTATATGACTCACCTTGTTTTTCGCAGCAAGGTGTTTTTAGG
     MHV HE   (680) TGTCTGGTTTGTTATATAATGTTTCCTGTATTGCCCAGCAGGGTGCATTTCTT
  Consensus  (1061) T AGTGGTTTGTTATATAATTCACCTTGTTTTTCGCAGCAAGGTGTTTTTAGG
```

FIGURE 3C (contd.)

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                                                                   Section 22
          (1114) 1114  ,1120      ,1130      ,1140      ,1150            1166
  HOBHEGA (1081) TATGATAATGTTAGCAGTGTCTGGCCTCTCTACCCCTATGGCAGATGTCCCAC
   BCV HE (1081) TATGATAATGTTAGCAGTGTCTGGCCTCTCTACCCTTATGGCAGATGCCCTAC
   MHV HE  (733) TATAATAATGTTAGTTCCTCTTGGCCAGCCTATGGGTACGGTCATTGTCGAAC
Consensus (1114) TATGATAATGTTAGCAGTGTCTGGCCTCTCTACCC TATGGCAGATGTCC AC

                                                                   Section 23
          (1167) 1167         ,1180      ,1190      ,1200            1219
  HOBHEGA (1134) TGCTGCTGATATTAATAACCCTGATTTACCCATTTGTGTGTATGATCCGCTAC
   BCV HE (1134) TGCTCCTGATATTAATACCCCTGATGTACCTATTTGTGTGTATGATCCGCTAC
   MHV HE  (786) GGCAGCTAACATTGGTTA---TATGGCACCTGTTTGTATCTATGACCCTCTCC
Consensus (1167) TGCTGCTGATATTAATAACCCTGATGTACCTATTTGTGTGTATGATCCGCTAC

                                                                   Section 24
          (1220) 1220      ,1230      ,1240      ,1250      ,1260       1272
  HOBHEGA (1187) CAGTTATTTTGCTTGGCATTCTTTTGGGCGTTGCGGTCATAATTATTGTAGTT
   BCV HE (1187) CAATTATTTTGCTTGGCATTCTTTTGGGTGTTGCGGTCATAATTATTGTAGTT
   MHV HE  (836) CGGTCATACTGCTAGGTGTGTTATTGGGTATAGCTGTGTTGATTATTGTGTTT
Consensus (1220) CAGTTATTTTGCTTGGCATTCTTTTGGGTGTTGCGGTCATAATTATTGTAGTT

                                                                   Section 25
          (1273) 1273   ,1280      ,1290      ,1300      ,1310          1325
  HOBHEGA (1240) TTGTTGTTATATTTTATGGTGGATAATGGTACTAGGCTGCATGATGCTTAGAC
   BCV HE (1240) TTGTTGTTATATTTTATGGTGGATAATGGTACTAGGCTGCATGATGCTTAGAC
   MHV HE  (889) TTGATGTTTTATTTTATGACGGATAGCGGTGTTAGATTGCATGAGGCATAA--
Consensus (1273) TTGTTGTTATATTTTATGGTGGATAATGGTACTAGGCTGCATGATGCTTAGAC

                                                                   Section 26
          (1326) 1326        1337
  HOBHEGA (1293) CATAATCTAAAC        SEQ ID NO: 9913
   BCV HE (1293) CATAATCTAAAC        SEQ ID NO: 9885
   MHV HE  (940) ------------        SEQ ID NO: 9896
Consensus (1326) CATAATCTAAAC
```

FIGURE 4

FIGURE 4A

```
                                                                    Section 1
                                     (1) 1          10         20                  39
  avian infectious bronchitis pol 1ab (1) ---------------------------------------
       bovine coronavirus pol 1ab     (1) MSKINKYGLELHWAPEFPWMFEDAEEKLDNPSSSEVDIV
      Human corona 229E pol 1ab       (1) -------------------MACNRVTLAVASDSEISANG
        Murine hepatitis pol 1ab      (1) MAKMGKYGLGFKWAPEEPWMLPNASEKLGNPERSEEDGF
                      Consensus       (1) MAKI KYGL    WAPEFPWM  NA EKL NPDSSE D
                                                                    Section 2
                                    (40) 40          50         60                 78
  avian infectious bronchitis pol 1ab (1) ---------------------------------------
       bovine coronavirus pol 1ab    (40) CSTTAQKLETGGICPENHVMVDCRKLLKQECCVQSSLIR
      Human corona 229E pol 1ab      (21) CSTIAQAVRRYSEAASNGFRACRFVSLDLQDCIVGIADD
        Murine hepatitis pol 1ab     (40) CPSAAQEPKVKGKTLVNHVRVNCSRLPALECCVQSAIIR
                      Consensus      (40) CST AQ LK   G      NHVRV C RLL LECCVQSAIIR
                                                                    Section 3
                                    (79) 79          90         100               117
  avian infectious bronchitis pol 1ab (1) ---------------------------------------
       bovine coronavirus pol 1ab    (79) EIVMNTRPYDLEVLLQDALQSREAVLVTEPLGMSLEACY
      Human corona 229E pol 1ab      (60) TYVMGLHGNQTLFCNIMKFSDRPFMLHG------WLVFS
        Murine hepatitis pol 1ab     (79) DIFVDEDPQKVEASTMMALQFGSAVLVKPSKRLSIQAWI
                      Consensus      (79) DIVM     PN LE   IMALQ R AVLV P    LSI AFS
                                                                    Section 4
                                   (118) 118         130        140               156
  avian infectious bronchitis pol 1ab (1) ----MASSLKQGVSPK------------PRDVILVSKDI
       bovine coronavirus pol 1ab   (118) VRGCNPNGWTMGLFRRRSVCNTGRCAVNKHVAYQLYMID
      Human corona 229E pol 1ab      (93) NSNYLLEEEDVVFGKR------GGGNVTYTDQYLCGADG
        Murine hepatitis pol 1ab    (118) NLGVLPKTAAMGLFKRVCLGNTRECSCDAHVAEHLFTVQ
                      Consensus     (118) N G LP SF MGLFKR  LCNTG CAV  HVAYLLF D
                                                                    Section 5
                                   (157) 157         170        180               195
  avian infectious bronchitis pol 1ab (24) REQLCDALFFYTSHNPKDYADALAVRQKFDRSLQTGKQF
       bovine coronavirus pol 1ab   (157) PAGVCFGAGQFVGWVIPLAFMPVQSRKFIVPWVMYLRKC
      Human corona 229E pol 1ab     (126) KPVMSEDLWQFVDHEGENEEIIINGHTYVCAWLTKRKPL
        Murine hepatitis pol 1ab    (157) PDGVCLGNGRFIGWFVPVTAIPEYAKQWLQPWSILLRKG
                      Consensus     (157) PDGVCDGLGQFVGWFIPL AIPINARQFI PWLI LKK
                                                                    Section 6
                                   (196) 196                 210      220           234
  avian infectious bronchitis pol 1ab (63) KFET------------VC--GLFLLKGVDKITPGVPAKV
       bovine coronavirus pol 1ab   (196) GEKG-AYNKDHKRGGFEHVYNFKVEDAYDLVHDEPKGKF
      Human corona 229E pol 1ab     (165) DYKR------------Q--NNLAIEEIEYVHGDALHTLR
        Murine hepatitis pol 1ab    (196) GNKGSVTSGHFRRAVTMPVYDFNVEDACEEVHLNPKGKV
                      Consensus     (196) GFKG          KRA  M VYNL VEDA DLVHDAPKGKF
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FIGURE 4A (contd.)

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                                                                  Section 7
                                      (235) 235  240        250       260       273
avian infectious bronchitis pol 1ab   (88)  LKATSKLADLEDIFGVSPLARKYRELLKTACQWSLTVEA
bovine coronavirus pol 1ab            (234) SKKAYALIRGYRGVKPLLYVDQYGCDYTGGLADGLEAYA
Human corona 229E pol 1ab             (190) NGSVLEMAKEVKTSSKVVLSDALDKLYKVFGSPVMTNGS
Murine hepatitis pol 1ab              (235) SCKAYALLKGYRGVKPILFVDQYGCDYTGCLAKGLEDYG
Consensus                             (235) SKKAYALAKGYRGVKPILLVDQYGCLYTGALA GLT YA
                                                                  Section 8
                                      (274) 274    280       290       300       312
avian infectious bronchitis pol 1ab   (127) LDVRAQTLDEIFDPTEILWLQVAAKIHVSSMAMRRLVGE
bovine coronavirus pol 1ab            (273) DKTLQEMKALFFIWSQELPFDVTVAWHVVRDPRYVMRLQ
Human corona 229E pol 1ab             (229) NILEAFIKPVFISALVQCTCGTKSWSVGDWTGFKSSCCN
Murine hepatitis pol 1ab              (274) DLTLSEMKELFPVWRDSLDSEVLVAWHVDRDPRAAMRLQ
Consensus                             (274) DITLAETKDLFPIWSD L  DV VAWHVDRDPRKAMRLQ
                                                                  Section 9
                                      (313) 313    320       330       340       351
avian infectious bronchitis pol 1ab   (166) VTAKVMDALGSNLSALFQIVKQQIARIFQKALAIFENVN
bovine coronavirus pol 1ab            (312) SASTIRSVAYVANPTEDLCDGSVVIKEPVHNYADDSIIL
Human corona 229E pol 1ab             (268) VISNKLCVVPGNVKPGDAVITTQQAGAGIKYFCGMTIKF
Murine hepatitis pol 1ab              (313) TLATVRCIDYVGQPTEDVVDGDVVVREPAHLLAANALVK
Consensus                             (313) VIATVRCVLYVNQPTEDLVDGSVVAREPIKLLAA SIV
                                                                  Section 10
                                      (352) 352    360       370       380       390
avian infectious bronchitis pol 1ab   (205) ELPQRLAALKMAFAKCARSLTVVVVERTLVVKEFAGTCL
bovine coronavirus pol 1ab            (351) RQHNLVDIMSCFYMEADAVVNAFYGVDLKDCGFVMQFGY
Human corona 229E pol 1ab             (307) VAN----------IEGVSVWRVIALQSVDCFVASSTEVE
Murine hepatitis pol 1ab              (352) RLP---RLVETMLYTDSSVTEFCYKTKLCECGFITQFGY
Consensus                             (352) RLPN I IL   FIEAASVI VIYL KL DCGFISQFGY
                                                                  Section 11
                                      (391) 391      400       410                429
avian infectious bronchitis pol 1ab   (244) ASINGAVAKTFEELPNGFMGSKIFTTLAFFKEAAVRVVE
bovine coronavirus pol 1ab            (390) IDCEQDLCDFKGWVPGNMIDGEACTTCGHVYETGDLLAQ
Human corona 229E pol 1ab             (336) EEHVNRMDTLCFNVRNSVTDECRLAMLGAEMTSNVRRQV
Murine hepatitis pol 1ab              (388) VDCCGDTCD-RGWVAGNMMDGFPCFGCTKNYMPWELEAQ
Consensus                             (391) IDC GDLCDFKGWVPNNMMDGF CTTLG  YESAVRLAQ
                                                                  Section 12
                                      (430) 430       440       450                468
avian infectious bronchitis pol 1ab   (283) NIPNAPRGTKGFEVVGNAKGTQVVVRGMRNDLTLLDQKA
bovine coronavirus pol 1ab            (429) SSGVLPVNPVLHTKSAAGYGGFGCKDSETLYGQTVVYFG
Human corona 229E pol 1ab             (375) ASGVIDISTGWFDVYDDIEAE--------SK-PWFVRKA
Murine hepatitis pol 1ab              (427) SSGVIPEGGVLETQSTDTVN----RESFKLYGHAVVPFG
Consensus                             (430) SSGVIPIGTVLFTVSADAFG    KDSFKLYG  VV KA
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FIGURE 4A (contd.)

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                                                                      Section 13
                                   (469) 469            480       490              507
avian infectious bronchitis pol 1ab (322) DIPVEPEGWSAILDGHLCYVFRSGDRFYAAPLSGNFALS
     bovine coronavirus pol 1ab (468) GCVYWS-PARNIWIPILKSSVKSYDGLVYTGVVGCKAIV
     Human corona 229E pol 1ab (405) EDIFGP-CWSALASALKQLKVTTGELVRFVKSICNSAVA
     Murine hepatitis pol 1ab (462) SAVYWS-PCPGMWLPVIWSSVKSYSGLTYTGVVGCKAIV
                    Consensus (469) D VYWS PWSAINIPIL SSVKSYDGL YTGVVGNKAIV
                                                                      Section 14
                                   (508) 508             520       530             546
avian infectious bronchitis pol 1ab (361) DVHCCERVVCLSDGVTPEINDGLILAALYSSFSVSELVT
     bovine coronavirus pol 1ab (506) KETNLICKALYIDYVQHKCGNLHQRELLGVSDVWHKQLL
     Human corona 229E pol 1ab (443) VVGGTIQILASVPEKFLNAFDVFVTATQTVFLCAVETCT
     Murine hepatitis pol 1ab (500) QETDAICRSLYMDYVQHKCGNLEQRALLGLDDVYHRQLL
                    Consensus (508)  VT  ICRSLYLDYVQHKCGNL QRAILGVSDVWHEQLT
                                                                      Section 15
                                   (547) 547             560       570             585
avian infectious bronchitis pol 1ab (400) ALKKGEPFKFLGHKFVYAKDAAVSFTLAKAATIADVLRL
     bovine coronavirus pol 1ab (545) LNRGVYKPLLENIDYENMRRAKFSLETETVCADGFMRFL
     Human corona 229E pol 1ab (482) IAGKAFDKVFDYVLLDNALVKLVITKLKGVRERGLNKVK
     Murine hepatitis pol 1ab (539) VNRGDYSLILENVDLFVKRRAEFACK-FATCGDGLVPLL
                    Consensus (547) INRKAY  LLENVDLFNARRA VS KLFAVCADGLVPLL
                                                                      Section 16
                                   (586) 586             600       610             624
avian infectious bronchitis pol 1ab (439) FQSARVIAEDVWSCFTEKSFEFWKLAYGKVRNLEEFVKT
     bovine coronavirus pol 1ab (584) LDDLVPRAYYLAVSGQAFCDYADKICHAVVSKSKELLDV
     Human corona 229E pol 1ab (521) YATVVVGSTEEVKSS------RVERSTAVLTIANNYSKL
     Murine hepatitis pol 1ab (577) LDGLVPRSYYLIKSGQAFTSMMVNFSHEVTDMCMDMALL
                    Consensus (586) LDSLVVRAYYLIKSGQAFS   VKISHAVVSIA EMSKL
                                                                      Section 17
                                   (625) 625  630          640       650             663
avian infectious bronchitis pol 1ab (478) YVCKAQMSIVILAAVLGEDIWHLVS---QVIYKLGVLFT
     bovine coronavirus pol 1ab (623) SLDSLSAALHYLNSKIVDLAQHFSDFGTSFVSKIVHFFK
     Human corona 229E pol 1ab (554) FDEGYTVVIGDVAYFVSDGYERLMASPNSVLITAVYKPL
     Murine hepatitis pol 1ab (616) EMHDVKVATKYVKKVTGKLAVRFKALGVAVVRKITEWED
                    Consensus (625) FLD LSVAI YLAAVIGDLAFRLMA G SVVSKIVHFF
                                                                      Section 18
                                   (664) 664  670          680       690             702
avian infectious bronchitis pol 1ab (514) KVVDFCDKHWKGFCVQLKRAKLIVTETFCVLKGVAQHCF
     bovine coronavirus pol 1ab (662) TFTTSTALATAWVLFHVLHGAYIVVESDIYFVKNIPRYA
     Human corona 229E pol 1ab (593) FAFNVNVMGTRPEKFPTTVTCENLESAVLFVNDKITEFQ
     Murine hepatitis pol 1ab (655) LAVDIAASAAGWLCYQLVNGLFAVANGVITFVQEVPELV
                    Consensus (664)  AVDI ALAFKWLCFQLL G FIV EAVIFFV  IPEF
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FIGURE 4A (contd.)

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                                                                 Section 19
                                    (703) 703       710         720          730        741
avian infectious bronchitis pol 1ab (553) QLLLDAIHSLYKSFKKCALGRTHGDLLFWKGGVHKIVQD
       bovine coronavirus pol 1ab (701) SAVAQAFRSVAKVVLDSLRVTFIDGLSCFKIGRRRICLS
     Human corona 229E pol 1ab (632) LDYSIDVIDNEIIVKPNISLCVPLYVRDYVDKWDDFCRQ
       Murine hepatitis pol 1ab (694) KNFVDKEKAFFKVLIDSMSVSIISGLTVVKTASNRVCLA
                          Consensus (703)  VSDAFKSLFKVVKDSISVSII GLS FK G  RICLA
                                                                 Section 20
                                    (742) 742       750         760         770         780
avian infectious bronchitis pol 1ab (592) GD-------------------------------------
       bovine coronavirus pol 1ab (740) GSKIYEVERGLLHSSQLPLIDVYDLTMPSQVQKAKQKPIY
     Human corona 229E pol 1ab (671) YS-------------------------------------
       Murine hepatitis pol 1ab (733) GSKVYEVVQKSISAYVMPVGCSHAICLVGEIEP------
                          Consensus (742) GSKIYEV     L A  LPL   D T
                                                                 Section 21
                                    (781) 781       790        800                  819
avian infectious bronchitis pol 1ab (594) ----------EIWFDAIDSVDVEDLGVVQEKSIDFEVCD
       bovine coronavirus pol 1ab (779) LKGSGSDFSLADSVVEVVTTSLTPCGYSEPPKVADKICI
     Human corona 229E pol 1ab (673) ---------NESWFEDDYRAFISVLDITDAAVKAAESKA
       Murine hepatitis pol 1ab (766) -------AVFEDDVVDVVKAPLTYQGCCKPPTSFEKICI
                          Consensus (781)           EDWVVDVVSA LT LGISDPPSIADKICI
                                                                 Section 22
                                    (820) 820       830        840                  858
avian infectious bronchitis pol 1ab (623) DVTLPENQPGHMVQIEDDGKNYMFFRFKKDENIYYTPMS
       bovine coronavirus pol 1ab (818) VDNVYMAKAGDKYYPVVVDG-HVGLLDQAWRVPCAGRRV
     Human corona 229E pol 1ab (703) FVDTIVPPQPSILKVIDGGKIWNGVIKNVNSVRDWLKSL
       Murine hepatitis pol 1ab (798) VDKLYMAKCGDQFYPVVVDNDTVGVLDQCWRFPCAGKKV
                          Consensus (820) VV LYMAKCGDIVYPVVVGK WVGVLDQ WRVPCAGKKV
                                                                 Section 23
                                    (859) 859       870        880                  897
avian infectious bronchitis pol 1ab (662) QLGAINVVCKAGGKTVTFGETTVQEIPPPDVVPIKVSIE
       bovine coronavirus pol 1ab (856) TFKEQPTVNEIASTPKTIKVFYELDKDTNTILNTACGVF
     Human corona 229E pol 1ab (742) KLNLTQQGLLGTCAKRFKRWLGTLLEAYNAFLDTVVSTV
       Murine hepatitis pol 1ab (837) EFNDKPKVRKIPST-RKIKLTFALDATFDSVLSKACSEE
                          Consensus (859)  LND P V KIAST RTIKITFILD  FNSVL TAVSIF
                                                                 Section 24
                                    (898) 898           910        920              936
avian infectious bronchitis pol 1ab (701) CCGEPWN--------TIFKKAYKEPIEVDTDLTVEQLLSV
       bovine coronavirus pol 1ab (895) EVDDTVDMEEFYAVVIDAIEEKISPCKELEGVGAKVSAF
     Human corona 229E pol 1ab (781) KIGG--------------LTFKTYAFDKPYIVIRDIVCKV
       Murine hepatitis pol 1ab (875) EVDKDVTLDELLDVVLDAVESTLSPCKEHDVIGTKVCAL
                          Consensus (898) EVGD V LDE    VVIDAIE TLSPCKEHDVIGDKVCAV
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FIGURE 4A (contd.)

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                                              Section 25
                                    (937) 937          950       960            975
   avian infectious bronchitis pol 1ab (733) IYEKMCDDLK-FPEAPEPPPFENVALVDKNGKLLDCIKS
            bovine coronavirus pol 1ab (934) LQKLEDNSLFLFDEAGEEVLASKLYCAFTAPEDDDFLEE
          Human corona 229E pol 1ab (807) ENKTEAEWIELFPHNDRIKSFSTFESAYMPIADPTHFDI
           Murine hepatitis pol 1ab (914) LDRLAGDYVYLFDEGGDEVIAPRMYCSFSAPDDEDCVAA
                              Consensus (937) LNKLEADWLFLFPEAGEEVIFSKLYCAFSAPDDDDCIDA
                                              Section 26
                                    (976) 976           990       1000          1014
   avian infectious bronchitis pol 1ab (772) CHLIYFDYESDDDIEEEDAEECDTDSGEAEECDTN----
            bovine coronavirus pol 1ab (973) SGVEEDDVEGEETDLLVTSAGEPCVASEQEE--------
          Human corona 229E pol 1ab (846) EEVELLDAEFVEPGCGGTLAVIDEHVFYKKDGVYYP---
           Murine hepatitis pol 1ab (953) DVVDADENQDDDAEDSAVIVADTQEEDGVAKGQVEADSE
                              Consensus (976) D VE DD E DD DDSAILAADD DA E EEG
                                              Section 27
                                   (1015) 1015 1020          1030       1040        1053
   avian infectious bronchitis pol 1ab (807) --------SECEELDEDTKVLALIQDPASIKYPLPLDED
           bovine coronavirus pol 1ab (1004) ----SSEILEDTLDDGPCVETSDSQVEEDVEMSDFADLE
          Human corona 229E pol 1ab (882) -----SNGTNILPVAFTKAAGGKVSFSDDVEVKDIEPVY
           Murine hepatitis pol 1ab (992) ICVAHTGSQEELAEPDAVGSQTPLASAEETEVGEASDRE
                             Consensus (1015)      S  SEDL EDD  AA A IQ AEDVEV D ADLE
                                              Section 28
                                   (1054) 1054  1060       1070       1080        1092
   avian infectious bronchitis pol 1ab (838) YSVYNGCIVHKDALDVVNLPSG-----------------
           bovine coronavirus pol 1ab (1039) SVIQDYENVCFEFYTTEP---------------------
          Human corona 229E pol 1ab (916) RVKLCFEFEDEKLVDVCEKAIG-----------------
           Murine hepatitis pol 1ab (1031) GIAEAKATVCADAVDACPDQVEAFEIEKVEDSILDELQT
                             Consensus (1054)  VI  FE VC DAVDVCP  IG
                                              Section 29
                                   (1093) 1093   1100       1110       1120        1131
   avian infectious bronchitis pol 1ab (860) -------------------------------------EE
           bovine coronavirus pol 1ab (1057) ----------------------------------EFVKV
          Human corona 229E pol 1ab (938) -----------------------------------KKIK
           Murine hepatitis pol 1ab (1070) ELNAPADKTYEDVLAFDAVCSEALSAFYAVPSDETHFKV
                             Consensus (1093)                                      KV
                                              Section 30
                                   (1132) 1132    1140       1150       1160        1170
   avian infectious bronchitis pol 1ab (862) TFVVN-----------------NCFEGAVKPLPQKVVDV
           bovine coronavirus pol 1ab (1062) LDLYVPKATRNNCWLRSVLAVMQKLPCQFKDKNLQDLWV
          Human corona 229E pol 1ab (942) HEG---------DWDSFCKTIQSALSVVSCYVNLPTYYI
           Murine hepatitis pol 1ab (1109) CGFYSPAIERTNCWLRSTLIVMQSLPLEFKDLEMQKLWI
                             Consensus (1132)  DLY P    R NCWLRS L VMQALPL FKDLNLQ LWV
```

FIGURE 4A (contd.)

Section 31

		1171 ,1180 ,1190 1209
	(1171)	
avian infectious bronchitis pol 1ab	(884)	LGDWGEAVDAQEQLCQQEPLQHTFEEPVENSTGSSKTMT
bovine coronavirus pol 1ab	(1101)	LYKQQYSQLFVDTLVNKTLANTVVPQGGYVADFAYWELT
Human corona 229E pol 1ab	(972)	YDEEGGNDLSLPVMISEWFLSVQQAQQEATLPDIAEDVV
Murine hepatitis pol 1ab	(1148)	SYKAGYDQCFVDKLVKSVFKSIILPQGGYVADFAYFFLS
Consensus	(1171)	LYK GYAQLFVD LVN IPLSIILPQGGYVADFAYFFLT

Section 32

		1210 ,1220 ,1230 1248
	(1210)	
avian infectious bronchitis pol 1ab	(923)	EQVVVEDQELPVVEQLQDVVVYTPTDLEVAKETAEEVDE
bovine coronavirus pol 1ab	(1140)	LCDWQCVAYWKCIKCLLALKLKGLDAMFFYGDVVSHVCK
Human corona 229E pol 1ab	(1011)	DQVEEVNSIEDIETV-VKHDVSPFEMPEEELNGLKILKQ
Murine hepatitis pol 1ab	(1187)	QCSFKAYANWRCLECDMELKLQGLDAMFFYGDVVSHMCK
Consensus	(1210)	DQVF A WKCIECDLDLKL GLDAMFFYGDVVSHVCK

Section 33

		1249 ,1260 ,1270 1287
	(1249)	
avian infectious bronchitis pol 1ab	(962)	FILIFAVPKEEVVSQKDGAQIKQEPIQVVKPQRE-KK--
bovine coronavirus pol 1ab	(1179)	CGESMVLIDVDVPETAHFALKDKLFCAFITKRSVYKAAC
Human corona 229E pol 1ab	(1050)	LDNNCWVNSVMLQIQLTGILDGDYAMQFFKMG-------
Murine hepatitis pol 1ab	(1226)	CGNSMTLLSADIPYTLHFGVRDDKFCAFYIPRKVFRAAC
Consensus	(1249)	CGNSM LISVDVPFTLHGALKDD FCQFVTPRKVFKAAC

Section 34

		1288 ,1300 ,1310 1326
	(1288)	
avian infectious bronchitis pol 1ab	(998)	-------------------------------------AK
bovine coronavirus pol 1ab	(1218)	VVDVNDSHSMAVVDGKQIDDHRVTSITSDKFDEIIGHGM
Human corona 229E pol 1ab	(1082)	---------------------------------------
Murine hepatitis pol 1ab	(1265)	AVDVNDCHSMAVVEGKQIDGKVVTKFIGDKFDFMVGYGM
Consensus	(1288)	VDVND HSMAVVDGKQID VT DKFDFIIGHGM

Section 35

		1327 ,1340 ,1350 1365
	(1327)	
avian infectious bronchitis pol 1ab	(1000)	KFKVKPATCEKPKFLEYKTCVGDLTVVEAKALDEFKEFC
bovine coronavirus pol 1ab	(1257)	SFSMITFEIAQLYCSCITPNVCFVKGDIIKVSKRVKAEV
Human corona 229E pol 1ab	(1082)	-------RVAKMIERCYTAEQCIRGAMGDVGLCMYRLLK
Murine hepatitis pol 1ab	(1304)	TFSMSPFELAQLYGSCITPNVCFVKGDVIKVVRLVNAEV
Consensus	(1327)	SFSMSPFEIAQLYGSCYTPNVCFVKGDIIKVLKLVKAEV

Section 36

		1366 ,1380 ,1390 1404
	(1366)	
avian infectious bronchitis pol 1ab	(1039)	LVNAANEHDTHGSGVAKATADFCCLDFVEYCEDYVKKHG
bovine coronavirus pol 1ab	(1296)	VVNPANGHMAHGGGVAKATAVAAGQQFVKETTDMVKSKG
Human corona 229E pol 1ab	(1114)	DLHTGFMVIDYKCSCTSGRLEESGAVLFCTPTKKAFPYG
Murine hepatitis pol 1ab	(1343)	IVNPANGRLAHGAGVAGATAEKACSAFIKETSDMVKAQG
Consensus	(1366)	IVNPANGHMAHGAGVAKAIAE AGA FVKETTDMVKAHG

FIGURE 4A (contd.)

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                                                                  Section 37
                                        (1405) 1405  1410      1420       1430          1443
 avian infectious bronchitis pol 1ab (1078) PQQR-----------------------------------
        bovine coronavirus pol 1ab (1335) VCATGDCYVSTGGKLCKTVLNVVGPDARTQGKQSYALLE
       Human corona 229E pol 1ab (1153) TCLNCNAPRMCTIRQLQGTIIEVQQKPEP-------VNP
        Murine hepatitis pol 1ab (1382) VCQVGECYESAGGKLCKKVLNIVGPDARGHGKQCYSLLE
                       Consensus (1405) VCQ GDCY S GGKLCK VLNIVGPDAR  GKQ YALLE
                                                                  Section 38
                                        (1444) 1444  1450      1460       1470          1482
 avian infectious bronchitis pol 1ab (1082) -----------LVTPSFVKGIQCVNNVVGPRHGDNNLHE
        bovine coronavirus pol 1ab (1374) RVYKHINKYDCVVTTLISASTFSVPSDVSLTYLLGTAEK
       Human corona 229E pol 1ab (1185) VSFVVKPVGSSIFRGAVSCGHYQTNIYSQNLCVDGFGVN
        Murine hepatitis pol 1ab (1421) RAYQHINKCDNVVTTLISAGIFSVPTDVSLTYLLGVVTK
                       Consensus (1444) RAY HINKCD VVTTLISAGIFSVPSDVSLTYLLG L K
                                                                  Section 39
                                        (1483) 1483  1490      1500       1510          1521
 avian infectious bronchitis pol 1ab (1110) KLVAAYKNVLVDGVVNYVVPVLSLGIFSVDFKMSIDAMR
        bovine coronavirus pol 1ab (1413) QVVLVSNNQEDEDLISKCQIIAVEGTKKLAERLSFNVGR
       Human corona 229E pol 1ab (1224) KIQPWTN--------DALNTICIKDADYNAKMEISVTPIK
        Murine hepatitis pol 1ab (1460) NVILVSNNQDDFDVIEKCQVTSVAGTKALSLQLAKNLCR
                       Consensus (1483) KVVLVSNNQDDFDVIAKCQITLVDGTKALALKLSINLIR
                                                                  Section 40
                                        (1522) 1522    1530      1540       1550          1560
 avian infectious bronchitis pol 1ab (1149) EAFEGCTIRVLLFS-------------------------
        bovine coronavirus pol 1ab (1452) SIVYETDANKLILSNDVAHVSTFNVIQDVLSLRHDIALD
       Human corona 229E pol 1ab (1256) NTVDTTPKEEFVVKEKLNAFLVHDNVAFYQGDVDTVVNG
        Murine hepatitis pol 1ab (1499) DVKFVTNACSSLFS-ESCEVSSYDVIQEVEALRHDIQLD
                       Consensus (1522) DIVF T A  LLFS DL FVSSHDVLQDV ALRHDI LD
                                                                  Section 41
                                        (1561) 1561      1570      1580                    1599
 avian infectious bronchitis pol 1ab (1163) ---------------------------------------
        bovine coronavirus pol 1ab (1491) DDARTFVQSNVDVVPEGWRVVNKFYQINGVRTVKYFECP
       Human corona 229E pol 1ab (1295) VDFDFIVNAANENLAHGGGIAKALDVYTKGKLQRLSKEH
        Murine hepatitis pol 1ab (1537) DDARVEVQANMDCLPTDWRLVNKFDSVDGVRTIKYFECP
                       Consensus (1561) DDAR FVQANMD LP GWRLVNKFD I GVRTIKYFECP
                                                                  Section 42
                                        (1600) 1600      1610      1620                    1638
 avian infectious bronchitis pol 1ab (1163) --ISQEHID----------------YFD--VTCKQKTLY
        bovine coronavirus pol 1ab (1530) GGIDICSQDKVFGYVQQGSFNKATVAQIKALFLDKVDIL
       Human corona 229E pol 1ab (1334) IGLAG--------------KVKVGTGVMVECDSLRIFNVV
        Murine hepatitis pol 1ab (1576) GGIFVSSQGKKFGYVQNGSFKEASVSQIRALLANKVDVL
                       Consensus (1600) GGIAI SQDK FGYVQNGSFK ATVAQIKALSLKKVDIL
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FIGURE 4A (contd.)

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                                             Section 43
                                 (1639) 1639       1650       1660                1677
avian infectious bronchitis pol 1ab (1182) LTEDGVKYRSIVLKPGDSLGQFG-----------QVYAKN
        bovine coronavirus pol 1ab (1569) DTVDGVNFTNRFVPVGESFGKSLGNVFCDGVNVTKHKCD
        Human corona 229E pol 1ab (1360) GFRKEKHERDLLIKAYNTINNEQGTPLTPILSCGIFGIK
         Murine hepatitis pol 1ab (1615) CTVDGVNFRSCCVAEGEVFGKTLGSVFCDGINVTKVKCS
                        Consensus (1639) LTVDGVNFRSILVK GESFGKSLGSVFCDGINVTKHKC

                                             Section 44
                                 (1678) 1678        1690       1700                1716
avian infectious bronchitis pol 1ab (1211) KIVFTADDVEDKEILYVPTTDKSILEYYGLDAQKYVIYL
        bovine coronavirus pol 1ab (1608) INYKGKVFFQFDNLSSEDLKAVRSSFNFDQKELLAYYNM
        Human corona 229E pol 1ab (1399) LETSLEVLLDVCNTKFVKVFVYTDTEVCKVKDFVSGLVN
         Murine hepatitis pol 1ab (1654) AIYKGKVFFQMSDLSFADIVAVKDAFGFDEPQLLKYYTM
                        Consensus (1678) IIYKGKVFFQF NLSEVDL AVSDSF FDLKDLLAYY M

                                             Section 45
                                 (1717) 1717         1730       1740               1755
avian infectious bronchitis pol 1ab (1250) QTLAQKWNVQYRDNELLILEWRDGNCWISSAIVLLQAAKI
        bovine coronavirus pol 1ab (1647) LVNCSKWQVVFNGKYFIFKQANNNCFVNVSCLMLQSINL
        Human corona 229E pol 1ab (1438) VQKVEQPKIEPK-PVSVIKVAPKPYRVDGKFSYFTEDLL
         Murine hepatitis pol 1ab (1693) LG-MCKWSVVVCGNYFAFKQSNNNCYINVACLMLQHLSL
                        Consensus (1717) L  M KWNVVFKGNYFIFKQANNNCFINVACLMLQAL L

                                             Section 46
                                 (1756) 1756          1770       1780              1794
avian infectious bronchitis pol 1ab (1289) REKGHLTEAWAKLLG-GDPTDEVAWCYASCTAKVGDFSD
        bovine coronavirus pol 1ab (1686) KFKIVQWQEAWLEFRSGRPARFVSLVLAKGGFKFGDPAD
        Human corona 229E pol 1ab (1476) CVADDKPIVLFTDSMLTLDDRGLALDNELSGVLSAAIKD
         Murine hepatitis pol 1ab (1731) KFPKWQWQEAWNEFRSGKPLRFVSLVLAKGSFKFNEPSD
                        Consensus (1756) KFK FQWQEAW EFRSGKP RFVALVLAKGGFKFGDPSD

                                             Section 47
                                 (1795) 1795  1800       1810       1820            1833
avian infectious bronchitis pol 1ab (1327) ANWLDANIAEHFDADYTNAFEKKRVSENCGIKS------
        bovine coronavirus pol 1ab (1725) SRDFLRVVFSQVDLTGAICDFEIACKCGVKQEQRTGVDA
        Human corona 229E pol 1ab (1515) CVDINKATP-SGNLIKFDIGSVVVYM-VVPSD--KDKHL
         Murine hepatitis pol 1ab (1770) SIDFMRVVLREADLSGATCNLEFVCKCGVKQEQRKGVDA
                        Consensus (1795) SIDFLRVV    ADLSGA C LEIVCKCGVKQEQRKGVDA

                                             Section 48
                                 (1834) 1834  1840        1850       1860           1872
avian infectious bronchitis pol 1ab (1360) --YELRG-----LEACIQPVRATNLLHFKTQYSNCPTCGA
        bovine coronavirus pol 1ab (1764) VMHFGTLSREDLEIGYTVDCSCGKKLIHCVRFDVPFLIC
        Human corona 229E pol 1ab (1551) DNNVQRCTRKLNRLMCDIVGTIPADYILPLVLSSLTCNV
         Murine hepatitis pol 1ab (1809) VMHFGTLDKGDLVRGYNIACTCGSKLVHCTQFNVPFLIC
                        Consensus (1834) VMHFGTLSR DLEIGYNIVCTCGAKLIHCL FNVPTLIC
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FIGURE 4A (contd.)

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                                                                                    Section 49
                                  (1873) 1873     1880               1890              1900          1911
avian infectious bronchitis pol 1ab (1393) NNTDRVIEASLPYLLLFATDGPATVDCDEDAVGTVVFVG
        bovine coronavirus pol 1ab (1803) SNTPASVKLPKGVGSANTFKGDKVGHYVHVKCEQSYQLY
      Human corona 229E pol 1ab (1590) SFVGELKAAEAKVITIKVTEDGVNVHDVTVTTDKSEEQQ
          Murine hepatitis pol 1ab (1848) SNTPEGRKLPDDVVAANIFTGGSVGHYTHVKCKPKYQLY
                         Consensus (1873) SNTPELRKLP  VISANITDGGAVVHYVHVKCD SYQLY
                                                                                    Section 50
                                  (1912) 1912      1920               1930              1940          1950
avian infectious bronchitis pol 1ab (1432) STNSGHCYFQAAKQAFDNLAKDRKFGKKSPYITAMYTRF
        bovine coronavirus pol 1ab (1842) DASNVKKVTDVT-NLSDCLYLKNLKQTFKSVLTTYYLDD
      Human corona 229E pol 1ab (1629) VGVIADKDKDLSSAMPSDTNTSELLTKAIDVDWVEFYCF
          Murine hepatitis pol 1ab (1887) DACNVNKVSEAKSNFEDCLYLKNLKQTFSSVLTTFYLDD
                         Consensus (1912) DA NV KVTDASGNLSDCLYLKNLKQTFSSVLTTFYLDF
                                                                                    Section 51
                                  (1951) 1951      1960               1970                          1989
avian infectious bronchitis pol 1ab (1471) AFKNETSLPVAKQSKGKSKSVKEDVSNLATSSKASFDNL
        bovine coronavirus pol 1ab (1881) VKKTEYNPDLSQYYCDGGKYYTQRIIKAQFKTFEKVDGV
      Human corona 229E pol 1ab (1668) KDAVTFATVDHSAFAYESAVVNGIRVLKTSDNNCWVNAV
          Murine hepatitis pol 1ab (1926) VKCVEEKPDLSQYYCESGKYYTKPIIKAQFRTFEKVDGV
                         Consensus (1951) VKKVEYAPDLSQYYCD SKYYT  IIKAQFKTFEKVDGV
                                                                                    Section 52
                                  (1990) 1990           2000              2010                      2028
avian infectious bronchitis pol 1ab (1510) TDFEQWYDSNIYDSLKVQESPDNFDKYVSFTTKEDSKLP
        bovine coronavirus pol 1ab (1920) YTNFKLIGHTICDILNAKLGFDSSKEFVEYKVTEWPTAT
      Human corona 229E pol 1ab (1707) CIALQYSKPHFISQGLDAAWNKFVLGDVEIFVAFVYYVA
          Murine hepatitis pol 1ab (1965) YTNFKLVGHSIADKLNAKLGFDCNSPFVEYKITEWPTAT
                         Consensus (1990) YTNFQLIGHSI E LNAKLGFD    FVEYKVTEWPTAT
                                                                                    Section 53
                                  (2029) 2029           2040              2050                      2067
avian infectious bronchitis pol 1ab (1549) LTLKVRGIKSVVDFRSKDGFIYKLTPDTDENSKAPVYYP
        bovine coronavirus pol 1ab (1959) GDVVLATDDLYVKRYERGCITFGKPVIWLSHEQASLNSL
      Human corona 229E pol 1ab (1746) RLMKGDKGDAEDTLTKLSKYLANEAQVQLEHYSSCVECD
          Murine hepatitis pol 1ab (2004) GDVVLASDDLYVSRYSSGCITFGKPVVWLGHEEASLKSI
                         Consensus (2029) GDVVLASDDLYVSRYSKGCITFGKPVVWLEHE ASL SL
                                                                                    Section 54
                                  (2068) 2068              2080              2090                   2106
avian infectious bronchitis pol 1ab (1588) VLDAISLKALWVEGNANFYVGHPNYYS------------
        bovine coronavirus pol 1ab (1998) TYFNRPLLVDENKFDVLKVDDVDDGG-------------
      Human corona 229E pol 1ab (1785) AKFKNSVASLNSAIVCASVKRDGVQVG------------
          Murine hepatitis pol 1ab (2043) TYFNRPSVVCENKFNVLPVDVSEPTDKGPVPAAVLVTGV
                         Consensus (2068) TYFNRSLLVIENKFNVL VD  D
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FIGURE 4A (contd.)

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                                                                              Section 55
                                 (2107) 2107          2120        2130             2145
avian infectious bronchitis pol 1ab (1615) -----------------------------------KSLHI
           bovine coronavirus pol 1ab (2024) ------------------------------DISESDAKES
          Human corona 229E pol 1ab (1812) -----------------------------------YCVHG
          Murine hepatitis pol 1ab (2082) PGADASAGAGIAKEQKACASASVEDQVVTEVRQEPSVSA
                          Consensus (2107)                              DI    SVHA

                                                                              Section 56
                                 (2146) 2146          2160        2170             2184
avian infectious bronchitis pol 1ab (1620) PTFWENAENFVKMGDKIGGVTMGLWRAEHLNKPNLERIF
           bovine coronavirus pol 1ab (2034) KEINIDKLSGVKKPFKVEDSVIVNDDTSEIKYVKSDSIV
          Human corona 229E pol 1ab (1817) --IKYYSRVRSVRGRALIVSVEQLEPCAQSRLLSGVAYT
          Murine hepatitis pol 1ab (2121) ADVKEVKLNGVKKPVKVEGSVVVNDPTSETKVVKSESIV
                          Consensus (2146)  DIKEIKLNGVKKP KIEGSVIVNDPTSESKLVKSLSIV

                                                                              Section 57
                                 (2185) 2185   2190        2200        2210            2223
avian infectious bronchitis pol 1ab (1659) NIAKKAEVSSSVTTTQCGKHIGKAAEFIADKVGGGVVRN
           bovine coronavirus pol 1ab (2073) DVYDMWLTGCRYVVRTANALSMAVNVETIRKFIKEGMTL
          Human corona 229E pol 1ab (1854) AFSGPVDKGHYTVYDTAKKSMYDGDRFVKHDLSLLSVTS
          Murine hepatitis pol 1ab (2160) DVYDMFLTGCKYVVWTANELSRLVNSPIVREYVKWGKGK
                          Consensus (2185) DVYDMFLTGCKYVV TANKLS   VNSPTIRKVIKFGVT

                                                                              Section 58
                                 (2224) 2224   2230        2240        2250            2262
avian infectious bronchitis pol 1ab (1698) ITDSEKGHCGITRG----------------------HFER
           bovine coronavirus pol 1ab (2112) VSIPIDLLNEREIKPVENVVKAVENKISACFNFIKWLFV
          Human corona 229E pol 1ab (1893) VVMVGGYVAPVNTVKPKPVINQLDEKAQKFFDFGDFLIH
          Murine hepatitis pol 1ab (2199) IVTPAKLLLLRDEKQEFVAPKVVKAKAIACYCAVKWFLL
                          Consensus (2224) IVIPIKLL LRD K   F VIK VK KA ACF FIKWLIL

                                                                              Section 59
                                 (2263) 2263     2270        2280        2290          2301
avian infectious bronchitis pol 1ab (1716) KMSPQFLKTLMFFFFVFLKAS----VKS----------V
           bovine coronavirus pol 1ab (2151) LLFGWIKISADNKVIYTTEVASKLTCKLVALAFKNAFLT
          Human corona 229E pol 1ab (1932) NFVIEFTWLLSMFTHCKTAVTTGDVKIMAKAPQRTGVVL
          Murine hepatitis pol 1ab (2238) YCFSWIKENTDNKVLYTTEVASKETFKLCCLAFKNALQF
                          Consensus (2263)  LF WIKFSLDNKVIYTTEVASKLT KL  LAFKNALLT

                                                                              Section 60
                                 (2302) 2302     2310        2320        2330          2340
avian infectious bronchitis pol 1ab (1741) VASYKTVLCKVVLATLLIVVEVVTSNPVMFTGIRVLDFL
           bovine coronavirus pol 1ab (2190) FKWSVVARGACLIATIFLLWFNFIYANVIFSDFYLPKIG
          Human corona 229E pol 1ab (1971) KRSLKYNLKASAAVLKSKWVLLAKFTKLLLLIYTLYSVV
          Murine hepatitis pol 1ab (2277) FNWSVVSRGFFLVATVFLLWFNFLYANVILSDFYLPNIG
                          Consensus (2302) FKWSVVARGA IIATIFLLWFNFIYANVILSDFYLP IG
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FIGURE 4A (contd.)

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                                                                        Section 61
                                   (2341) 2341     2350       2360               2379
avian infectious bronchitis pol 1ab (1780) FEGSLCGPYKDYG---------------KDSFDVLRYCA
        bovine coronavirus pol 1ab (2229) FLPTFVCKTAQWIKSTFSLVTTICDLYSIQDVGFKNQYCN
        Human corona 229E pol 1ab (2010) LLCVRFS---PFN---ECSETVN--GYAKSNFVKDDYCD
          Murine hepatitis pol 1ab (2316) PLPTFVSQIVAWFKTTFGVSTICDFYQVTDLGYRSSFCN
                         Consensus (2341) FLPTFVG I   W KSTF L TICD Y IKDLGFK   YCN
                                                                        Section 62
                                   (2380) 2380      2390       2400              2418
avian infectious bronchitis pol 1ab (1804) DDFTIRVVLHDKDSLHLYKKHAYSIEQVYKDAASGFIFNW
        bovine coronavirus pol 1ab (2268) GSIAGQFLLAGFDMHDNYKAHDVVQYEADRRAFVDYTGV
        Human corona 229E pol 1ab (2041) GGLGCKMCLFGYQEISQESHEDVVWKHTTDPLFSNMQPF
          Murine hepatitis pol 1ab (2355) GSMVCELCFSGFDMLDNYDAINVVQHVVDRRLSFDYISL
                         Consensus (2380) GSIICKLCLAGFDMLDNYKHIDVVQHVIDRRLS DY  V
                                                                        Section 63
                                   (2419) 2419       2430       2440              2457
avian infectious bronchitis pol 1ab (1843) NWLYIVFLLLFVKPVAG------FVI--ICYCVKYLVLN
        bovine coronavirus pol 1ab (2307) IKIVPELIVSYALYTAWFYPLFALIIQILTTWLPELFM
        Human corona 229E pol 1ab (2080) LVMVLLIIFG------------DNYLRCFLLYFVAQMIS
          Murine hepatitis pol 1ab (2394) FKLVVELVICYSLYTVCFYPLFVLIGMQLLTTWLPEFFM
                         Consensus (2419) IKLVLELIIGYALYTA FYPLF LIIIQILTTWLPELFM
                                                                        Section 64
                                   (2458) 2458        2470       2480              2496
avian infectious bronchitis pol 1ab (1874) STVLQTGVCFLDWLEVQTVFSHFNFMGAGFYFWLFYKLYI
        bovine coronavirus pol 1ab (2346) LSTLHWSVRLLVSLANMLPAHVFMRFYIIIASFIKLFIL
        Human corona 229E pol 1ab (2107) TVGVFLGYKETNWLLHFIEFDVICDELLVTVIVIKVISF
          Murine hepatitis pol 1ab (2433) IETMHWSARLFVEVANMLPAFTLLRFYIVVTAMYKVYCL
                         Consensus (2458) LSTLHWSVRLLVWFANMLPAHVILRFYIVI ALIKVI L
                                                                        Section 65
                                   (2497) 2497          2510       2520            2535
avian infectious bronchitis pol 1ab (1913) QVHHLLYCKDVTAEVCKRVASNFQEVSVVLGGRKQIVH
        bovine coronavirus pol 1ab (2385) FRHVAYGCSKPGCLFCYKRNRSLRVKCSTIVGGMIRYYD
        Human corona 229E pol 1ab (2146) VRHVLFGCENRDCIACSKSALLKRFPVNTIVNGVQRSFY
          Murine hepatitis pol 1ab (2472) CRHVMYGCSKPGCLFCYKRNRSVRVKCSTVVGGSLRYYD
                         Consensus (2497)  RHVIYGCSKPGCLFCYKRNRSLRVKVSTIVGGMIRYYD
                                                                        Section 66
                                   (2536) 2536          2550       2560            2574
avian infectious bronchitis pol 1ab (1952) NYTHSEYNFCKRHNWYCRNCDDYGHQNTFMSPEVAGETS
        bovine coronavirus pol 1ab (2424) VMANGGTGFCSKHQWNCIDCDSYKPGNTFITVEAALDLS
        Human corona 229E pol 1ab (2185) VNANGCSKFCKKHREFFCVDCDSYGYGSI-ITPEVSRETG
          Murine hepatitis pol 1ab (2511) VMANGGTGFCTKHQWNCLNCNSWKPGNTFITHEAAADLS
                         Consensus (2536) VMANGGTGFCKKHQWNCINCDSYKPGNTFITPEVAADLS
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FIGURE 4A (contd.)

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                                                                              Section 67
                                     (2575) 2575 2580      2590       2600        2613
avian infectious bronchitis pol 1ab (1991) EKTIRHVKFTAYDVHVVDEACLVDDFVNIKYKAATPGKD
      bovine coronavirus pol 1ab (2463) KELKRPIQTPDVAYHTVTDVKQVGCYMRLFYERDGQRTY
      Human corona 229E pol 1ab (2224) NITATNVQSIGPAAVMIDKVEFENGFYRLYSCETFWRYN
       Murine hepatitis pol 1ab (2550) KELKRPVNIIDSFVYSVTEVKQVGCSMRLFYERDGQRVY
                       Consensus (2575) KELKRPVQPTD AYHTVTEVKQVGCFMRLFYERDGQR Y
                                                                              Section 68
                                     (2614) 2614 2620      2630       2640        2652
avian infectious bronchitis pol 1ab (2030) SASSAVKCFSVTDFLKKAVFLKEALKCEQISQDGFIVCN
      bovine coronavirus pol 1ab (2502) DDVNASLFVDYSNLLHSKVKGVPNMHVVVVEND------
      Human corona 229E pol 1ab (2263) FDITESKYSCKEVFKN---CNVLDDFTVFNNKG------
       Murine hepatitis pol 1ab (2589) DDVNASLTVDMNGLLHSKVKGVPETHVVVVENE------
                       Consensus (2614) DDVNASLFVDMS LLHSKVKGVPDLHVVVVEND
                                                                              Section 69
                                     (2653) 2653 2660      2670       2680        2691
avian infectious bronchitis pol 1ab (2069) TQSAHALEEAKNAAIYYAQYLCKPTIIIDQAIYEQLVVE
      bovine coronavirus pol 1ab (2535) ----ADKANFLNTAVFYAQSLFRPILMVDKNLITTANTG
      Human corona 229E pol 1ab (2293) ----TNVTQVKNSSVYFSQLLCRTIKTVDSEILSTLSVD
       Murine hepatitis pol 1ab (2622) ----ADKAGELGLAVFYAISLYRPMLMVEKKIITTANTG
                       Consensus (2653)     ADKANFLNAAVFYAQSLCRPILMVDK LITTLNVG
                                                                              Section 70
                                     (2692) 2692 2700      2710       2720        2730
avian infectious bronchitis pol 1ab (2108) PVS-KSVIDKVCSILSSLISVDTAATN------------
      bovine coronavirus pol 1ab (2570) TSVTETMFDVYVDTFLSMFDVDKKSINALIATAHSSIKQ
      Human corona 229E pol 1ab (2328) FNG--VLHKAVIDVLRNSIGKILN--------AN-----
       Murine hepatitis pol 1ab (2657) LSVSRTMFDLYVDSLLNVLDVDRKSLTSFVNAAHNSLKE
                       Consensus (2692)  SVSKTMFDLYVDTLLSIFDVDKKSLNA I  AH SIK
                                                                              Section 71
                                     (2731) 2731      2740      2750               2769
avian infectious bronchitis pol 1ab (2134) -----------YKAGTLRDALLSITKDEEAVDMAIFCHN
      bovine coronavirus pol 1ab (2609) GTQLCKVLDTFLSCARKSCSIDSDVDTKCLADSVMSAVS
      Human corona 229E pol 1ab (2352) -----------MSLAECKRAIGLSLSDHEFTSAISNAHR
       Murine hepatitis pol 1ab (2696) GVQIEQVMDTFIGCARRKCAIDSDVETKSITKSVMSAVN
                       Consensus (2731) G QI    VLDTFISCARKKCAIDSDVDTKEITDSVMSAVN
                                                                              Section 72
                                     (2770) 2770      2780      2790               2808
avian infectious bronchitis pol 1ab (2162) HDVDYTGDGFTNVIPSYGIDTGKLTPRDRGFLINADASI
      bovine coronavirus pol 1ab (2648) AGLELIDDESCNNLVPTYLKG-DNIVAADLGVLIQNSAKH
      Human corona 229E pol 1ab (2380) CDVLLSDLSFNNFVSSYAKPEEKLSAYDLACCMRAGAKV
       Murine hepatitis pol 1ab (2735) AGVDFTDESCNNLVPTYVKS-DTIVAADLGVLIQNNAKH
                       Consensus (2770) AGVDLTDESFNNLVPSYLK DKIVAADLGVLIQN AKH
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FIGURE 4A (contd.)

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                                                                          Section 73
                                      (2809) 2809       2820      2830              2847
avian infectious bronchitis pol 1ab (2201) ANLPVKNAP--PVVWKFSELIKLLDSCLKYLISATVKSG
          bovine coronavirus pol 1ab (2686) VQGNVAKIAGVSCIKSVDAFNQLLSDFQHKLKKACCKTG
          Human corona 229E pol 1ab (2419) VNANVLTKDQTPIVWHAKDFNSLSAEGRKYIVKTSKAKG
            Murine hepatitis pol 1ab (2773) VQANVAKAANVACIWSVDAFNQLEADLQHRLRKACSKTG
                            Consensus (2809) VNANVAKAANVPCIWSVDAFNQLSAD QKYLRKAC KTG
                                                                          Section 74
                                      (2848) 2848         2860      2870            2886
avian infectious bronchitis pol 1ab (2238) VREFILKSGAKQVIACHTQKLLVEKKAGGIVSGTFKCFK
          bovine coronavirus pol 1ab (2725) LKLKLTYNKQ--MANVSVLTTPFSLKGLAVFS-------
          Human corona 229E pol 1ab (2458) LTELLLINENQAVTQIPATSIVAKQGALDAGH-------
            Murine hepatitis pol 1ab (2812) LKIKLTYNKQ--EANVPILTTPFSLKGGAVFS-------
                            Consensus (2848) LKFKLTYNKQ  VANVPILTTPFSLKAGAVFS
                                                                          Section 75
                                      (2887) 2887           2900      2910            2925
avian infectious bronchitis pol 1ab (2277) SYFKWLLIFYILFTACCSGYYYMEVSKSFVHPMYDVNST
          bovine coronavirus pol 1ab (2755) ------YFMYVCELLSLVCFIGLWCLMPTYTVHKSDFQL
          Human corona 229E pol 1ab (2490) ------SLTWLWLLCGLVCLIQFMLCFFMPYFMYDIVSS
            Murine hepatitis pol 1ab (2842) ------RMLQWLFVANLICFIVLWALMPTYAVHKSDMQL
                            Consensus (2887)        ILYILFLA LVCFI LWLLMPTYHVMYSDMSL
                                                                          Section 76
                                      (2926) 2926             2940      2950          2964
avian infectious bronchitis pol 1ab (2316) LHVEGFLVIDKGVLREIVPEDTCFSHKEVNEDAEWGRPY
          bovine coronavirus pol 1ab (2788) PVYASYKVLDNLVIRDVSVEDVCFANKFEQFDQWYESTF
          Human corona 229E pol 1ab (2523) FEGYDFLYIENGQLKNFEAPLKCVRLVEENFEDWHYAKF
            Murine hepatitis pol 1ab (2875) PLYASFLVIDNLVLRDVSVTDACFANKFNQFDQWYESTF
                            Consensus (2926) PLYASFKVIDNGVLRDVSVED CFANKFENFDQWYESTF
                                                                          Section 77
                                      (2965) 2965 2970         2980      2990          3003
avian infectious bronchitis pol 1ab (2355) DNSRNCPIVTAVIDGDGTVATGVPGFVSWVMDGVMFIHM
          bovine coronavirus pol 1ab (2827) GLSYYSNSMA----CPIVVAVVDQDLGSTVFNVPTKVLR
          Human corona 229E pol 1ab (2562) GFTPLNK-Q------SCPIVVGVSEIVNTVAGIPSNVYL
            Murine hepatitis pol 1ab (2914) GLAYYRNSKA----CPVVVAVIDQDIGHTLFNVPTTVLR
                            Consensus (2965) GLSYY NSMA    CPIVVAVGVQDIVSTVFNVPT VLR
                                                                          Section 78
                                      (3004) 3004  3010         3020      3030          3042
avian infectious bronchitis pol 1ab (2394) TQTERKPWYIPTWFNREIVGYTQDSIITEGSEYTSIALF
          bovine coronavirus pol 1ab (2862) YGYHVLHFITHALSADGVQCYTPHSQISYSNFYASGCVL
          Human corona 229E pol 1ab (2594) VGKTLIFTLQAAFGNAGVCYDIFGVTTPEK------CIF
            Murine hepatitis pol 1ab (2949) YGFHVLHFITHAFATDSVQCYTPHMQIPYDNFYASGCVL
                            Consensus (3004) YGFHVLHFITHAFANDGVQCYTPHSQIPY NFYASGCVL
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FIGURE 4A (contd.)

Section 79

	(3043)	3043 3050 3060 3070 3081
avian infectious bronchitis pol 1ab	(2433)	SARSLYLTASNTPQLYCFNGDNDAPGALPFGSIIPHRVY
bovine coronavirus pol 1ab	(2901)	SSACTMFAMADGSPQPYQYTEGLMQNASLYSSLVPHVRY
Human corona 229E pol 1ab	(2627)	TSASTRLEGLGGN-NVYCYNTALMEGSLPYSSIQANAYY
Murine hepatitis pol 1ab	(2988)	SSLCTMLAHADGTPHPYCMTGGVMHNASLYSSLAPHVRY
Consensus	(3043)	SSACTMLAAADGSPNPYCYTDGLM NASPYSSIIPHVRY

Section 80

	(3082)	3082 3090 3100 3110 3120
avian infectious bronchitis pol 1ab	(2472)	FQPNG--VRLIVPQQILHTPYVGKFVSDSYCIGSV-EYT
bovine coronavirus pol 1ab	(2940)	NLANAKGFIRFPEVIREGLVRIVRTRSMSYCKVGLCEEA
Human corona 229E pol 1ab	(2665)	KYDNGNFIKLPEVIAQGFGFRTVRTIATKYCRVGECVES
Murine hepatitis pol 1ab	(3027)	NLASSNGVIRFPEVVSEGIVRVVRTRSMTYCRVGICEEA
Consensus	(3082)	NLANGNGVIRFPEVL EGIVRVVRTRSMSYCRVGLCEEA

Section 81

	(3121)	3121 3130 3140 3159
avian infectious bronchitis pol 1ab	(2509)	RPSYCVSLNPQWLLFNDEYTSKPCVFDCSTVRELMESMV
bovine coronavirus pol 1ab	(2979)	DEGICFNFNGSWVLNNDYYRSLPGTFCGRDVFDLIYQLF
Human corona 229E pol 1ab	(2704)	NAGVCFGEDKWEVNDG---RVANCYVCGTGLWNLVFNIL
Murine hepatitis pol 1ab	(3066)	DEGICFNFNRSWVLNNPYYRAMPGTFCGRNAFDLIHQVL
Consensus	(3121)	DEGICFNFNKSWVLNNDYYRSLPGTFCGR VFDLIFQIL

Section 82

	(3160)	3160 3170 3180 3198
avian infectious bronchitis pol 1ab	(2548)	STFFTGVNPN-IYMQLATMFLLLVVVVLIFAMVLIFQGV
bovine coronavirus pol 1ab	(3018)	KGLAQPVDELALTASSIAGAILAVIVVLVFYYLIKLKRA
Human corona 229E pol 1ab	(2740)	SMFSSSFSVAAMSGQILLNCALGAFAIFCCFLVTKFRRM
Murine hepatitis pol 1ab	(3105)	GGLVRPIDEFALTASSVAGAILAIIVVLAFYYLIKLKRA
Consensus	(3160)	SGLASPVDF ALTASSIAGAILAVIVVLIFYYLIKLKRA

Section 83

	(3199)	3199 3210 3220 3237
avian infectious bronchitis pol 1ab	(2586)	-KAYATTVFITMLVWVIVAFILCVHSYNSVLAVILLVLY
bovine coronavirus pol 1ab	(3057)	-GDYTSIVFVNVIVWCVNFMMLFVFQVYPTLSCVYAICY
Human corona 229E pol 1ab	(2779)	-GDLSVGVCTVVVAVLLNNVSYIVTQNLVTMIAYAILYF
Murine hepatitis pol 1ab	(3144)	-GDYTSVVVINVIVWCINFLMLFVFQVYPTLSCLYACFY
Consensus	(3199)	FGDYTSIVFINVIVWCINFLMLFVFQVYPTLSCIYAIFY

Section 84

	(3238)	3238 3250 3260 3276
avian infectious bronchitis pol 1ab	(2625)	CYASLVTSRNTVLIMHCWLVFTEGLIVPTWLACCILGFI
bovine coronavirus pol 1ab	(3096)	FYATLYFPSEISVIMHLQWLVMYGTIMPLWFCLLYISVV
Human corona 229E pol 1ab	(2818)	FATRSLR--YA-WIWCAAYLLAYISFAPWNLCAWLFLAM
Murine hepatitis pol 1ab	(3183)	FYTTLYFPSEISVVMHLQWLVMYGAIMPLWFCIIYVAVV
Consensus	(3238)	FYTTLYFPSEISVIMHLQWLVMYGSIMPLWLCIIYIAVV

FIGURE 4A (contd.)

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                                                                          Section 85
                                    (3277) 3277         3290      3300          3315
avian infectious bronchitis pol 1ab (2664) IYMYTPLFLWCYGTTKNTRKLYDGNEFVSNYDLAAKSTF
        bovine coronavirus pol 1ab (3135) VS------NHAFWVFAYCRRLGTSVRSDGTFEEMALTTF
      Human corona 229E pol 1ab (2854) LTGL----LPSLLKLKVSTNLFEGDKFVGTFESAMAGTF
          Murine hepatitis pol 1ab (3222) VS------NHALWLFSYCRKIGTEVRSDGTFEEMALTTF
                         Consensus (3277) VS      NHALWLFKYCRKLGTGVRSVGTFEEMALTTF
                                                                          Section 86
                                    (3316) 3316          3330      3340         3354
avian infectious bronchitis pol 1ab (2703) VIRGSEFVNITREIG-DKDEAYLSAYARLKYYEGTGSEQ
        bovine coronavirus pol 1ab (3168) MITKDSYCTLKKSLSDVAFNRYLSLYNKYRYYSGKMDTA
      Human corona 229E pol 1ab (2889) VIDMRSYEKLANSISPEKLKSLAASYNRYKLYSGHANEA
          Murine hepatitis pol 1ab (3255) M-TKESYCNLKNSVSDVAFNRTLSLINKYRYESCKMDTA
                         Consensus (3316) MITKDSYCKLKNSISDVKFNRYLSLYNKYKYYSGKMDTA
                                                                          Section 87
                                    (3355) 3355 3360      3370      3380        3393
avian infectious bronchitis pol 1ab (2741) DYLQECRAWLKYTLDQYR-NSGVEIVSTLPRYSIGVCRL
        bovine coronavirus pol 1ab (3207) AYREAACSQLAKAMDTFTNNNGSDVLYQPPTASVSTSFL
      Human corona 229E pol 1ab (2928) DYRCLCYAYLAKAMLDFSRDHN-DILYTPPTVSYG-STL
          Murine hepatitis pol 1ab (3294) AYREAACSQLAKAMETFNHNNGNDVLYQPPTASVTTSFL
                         Consensus (3355) DYREACCAQLAKAMDTFS NNG DILYTPPTASVGTSFL
                                                                          Section 88
                                    (3394) 3394  3400      3410      3420       3432
avian infectious bronchitis pol 1ab (2779) QSGFKVLVSPSSAVTKEIVSVSYRGNNFVGLWLGLTIYC
        bovine coronavirus pol 1ab (3246) QSGIVKMVNPTSKVEPCIVSVTYGNMTLNGLWLDDKVYC
      Human corona 229E pol 1ab (2965) DAGLRKMAQPSGFVSKQVVRVCLGNTVLNGLWLGDIVYC
          Murine hepatitis pol 1ab (3333) QSGIVKMVSPTSKVEPLIVSATYGNMTLNGLWLDDKVYC
                         Consensus (3394) QSGIVKMVSPSSKVEPCIVSVTYGNMTLNGLWLGDKVYC
                                                                          Section 89
                                    (3433) 3433   3440      3450      3460       3471
avian infectious bronchitis pol 1ab (2818) PRHVLGKFSGDQWNFVLNLANNHEFEVTTQHG--VTLNV
        bovine coronavirus pol 1ab (3285) PPHVICSASDMTNPDYTNLLCRVTSSDFTVLFDRLSLTV
      Human corona 229E pol 1ab (3004) PKHVTASN-TTSAIDYDHEYSIMRLHNESTISGTAFLGV
          Murine hepatitis pol 1ab (3372) PKHVICSSADMTDPDYPNLLCRVTSSDFCVMSGRMSLTV
                         Consensus (3433) PRHVICSASDMT PDY NLLCRVTSSDFTVISGRLSLTV
                                                                          Section 90
                                    (3472) 3472    3480      3490      3500       3510
avian infectious bronchitis pol 1ab (2855) VSRRLKSAVLILQTAVAHAELLKYKFIKANCCDSTTIAC
        bovine coronavirus pol 1ab (3324) MSYQMQGCMLVLTVTLQNSRIDKYTFGVVKPGETFTVLA
      Human corona 229E pol 1ab (3042) VGATMHGVTIKIKVSQTNMHTIRHSIRTLKSGEGFNILA
          Murine hepatitis pol 1ab (3411) MSYQMQGCQLVLTVTLQNPNLFKYSFGVVKPGETLTVLA
                         Consensus (3472) MSYQMQGCMLVLTVTLQNA TPKYSFGVVKPGETFTILA
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FIGURE 4A (contd.)

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                                               Section 91
                          (3511) 3511     3520      3530              3549
avian infectious bronchitis pol 1ab (2894) A-G-TVVGLYPVTMRSNGTIRA-FLAGA--SV-FNIEKG
           bovine coronavirus pol 1ab (3363) AYNGKPQGAFHVTMRSSYWIKGSFLCGSCGSVGYVLMGD
            Human corona 229E pol 1ab (3081) CYDGCAQGVEGYNMSTNWTIRGSYINGACGSPGYNLKNG
             Murine hepatitis pol 1ab (3450) AYNGRPQGAFHVTLRSSHTIKGSFLCGSCGSVGYVLTGD
                             Consensus (3511) AYNGKPQGAFHVTMRSSWTIKGSFLCGACGSVGYVL GG
                                               Section 92
                          (3550) 3550       3560      3570             3588
avian infectious bronchitis pol 1ab (2933) VYNFFMYHHLLLPNALHTGTDLMYHEYLGYVYEEVACRV
           bovine coronavirus pol 1ab (3402) CVKFVYMHQLELSTGCHTGTDFNGDFYGPYKDAQVVQLP
            Human corona 229E pol 1ab (3120) EYEFVYNIQIELGSGSHVGSSFDGVMYGGFEYQPNLCVE
             Murine hepatitis pol 1ab (3489) SVRFVYIHQLELSTGCHTGTDFSGNFYGPYRDAQVVQLP
                             Consensus (3550)  VKFVYMHQLELSTGCHTGTDF GDFYGPYKDAQVVQLP
                                               Section 93
                          (3589) 3589       3600      3610             3627
avian infectious bronchitis pol 1ab (2972) PPDNLVTNITYVWIYAALISVKESSFSLPKWLESTTVSV
           bovine coronavirus pol 1ab (3441) VQDYIQSVNFVANLYAAILNN-------CNWFVQSDKCSV
            Human corona 229E pol 1ab (3159) SANQMLTVKVVAWLYAAILNG-------CTWWLKGEKLFV
             Murine hepatitis pol 1ab (3528) VQDYTQTVNVVAWIYAAIFNR-------CNWFVQSDSCSL
                             Consensus (3589) VQDYIQTVNVVAWLYAAILN       CNWFLQSDKCSV
                                               Section 94
                          (3628) 3628         3640      3650           3666
avian infectious bronchitis pol 1ab (3011) DDYIKVLGDNYITPFSTSTAITKTSAITGVDVCKLLRTI
           bovine coronavirus pol 1ab (3474) EDFLVWALSNGFSQVKSDLVIDALASMTGVSLETLLAAI
            Human corona 229E pol 1ab (3192) EHYLEIAQANGFTAMNGEDAFSILAAKTGVCVERLLHAI
             Murine hepatitis pol 1ab (3561) EEFLVWAMTNGFSSIKADLVLDALASMTGVTVEQVLAAI
                             Consensus (3628) EDFNVWALSNGFSAIKADLVIDALAAMTGVSVEKLLAAI
                                               Section 95
                          (3667) 3667         3680      3690           3705
avian infectious bronchitis pol 1ab (3050) MVKNSQWGCDPTLGQYNFEDELTPESVFNQIGCVRLQS-
           bovine coronavirus pol 1ab (3513) KRLKNGFQLRQTMFSCSFEDDLTPSDVYQLLAGIKLQSK
            Human corona 229E pol 1ab (3231) QVLNNGFGGKQILGYSSLNDEFSINEVVKQMFGVNLQS-
             Murine hepatitis pol 1ab (3600) KRLHSGFQGKQILGSCVLEDDLTPSDVYQQLAGVKLQSK
                             Consensus (3667) KVLNSGFQGKQILGSCSLEDELTPSDVYQQLAGVKLQSK
                                               Section 96
                          (3706) 3706          3720      3730          3744
avian infectious bronchitis pol 1ab (3088) -SFVRKATS-WFWSRCVLACFLFVLCAIVLFTAVPLKFY
           bovine coronavirus pol 1ab (3552) RTRLVKGIVCWIMASTFLFSCIITAFVKWTMFMYVTTNM
            Human corona 229E pol 1ab (3269) -GKTTSMFKSISLFAGFFVMFWAELFVYTITIWVNPGFL
             Murine hepatitis pol 1ab (3639) RTRVIKGTCCWILASTFLFCSIISAFVKWTMFMYVTTHM
                             Consensus (3706) RTRVIKGT CWILASTFLFCFIISLFVKWTMFMYVTTFM
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FIGURE 4A (contd.)

Section 97

	(3745)	3745 3750 3760 3770 3783
avian infectious bronchitis pol 1ab	(3125)	VYAAVILLMAVLFISFTVLLVMAYMDTFLLPTLLTVLIG
bovine coronavirus pol 1ab	(3591)	LSITFCALCVISLAMLLVKHKHLYLTMYILPVLFTLLYN
Human corona 229E pol 1ab	(3307)	TPFMLLVALSLCLTFVVKLFKVLFLQVFLLISLIVAAIQ
Murine hepatitis pol 1ab	(3678)	IGVTLCALCFVSFAMLLLKHKHLYLTMYIMFVLCTLFYT
Consensus	(3745)	L ITICLLCLVSFAMLLVKHKHLYLTMFILPVLITLIYN

Section 98

	(3784)	3784 3790 3800 3810 3822
avian infectious bronchitis pol 1ab	(3164)	VCAEMPFIYNTLISQVVIFLSQWYDPVVFDTMVPWMFLP
bovine coronavirus pol 1ab	(3630)	NYLVVYKQTFRGYVYAWLSYYVPSVEYTYTDEVIYGMLL
Human corona 229E pol 1ab	(3346)	NCAWDYHVTKVLAEKFDYNVSVMQMDIQGFVNIFICLFV
Murine hepatitis pol 1ab	(3717)	NYLVVYKQSFRGLAYAWLSHFVPAVDYTYMDEVLYGVVG
Consensus	(3784)	NYLVVYKQTFRLIAYAWLSVSVPAVDYTY DEVIYGLLL

Section 99

	(3823)	3823 3830 3840 3850 3861
avian infectious bronchitis pol 1ab	(3203)	LVLYTAFKCVQGCYMNSFNTSLLMLYQFVKLGFVIYTSS
bovine coronavirus pol 1ab	(3669)	LIGMVFVTLRSINHD---------LFSFIMFVGRVISVV
Human corona 229E pol 1ab	(3385)	ALLHTWRFAKER---------------CTHWCTYLFSLI
Murine hepatitis pol 1ab	(3756)	LVAMVFVIMRSINHD---------VTSIMFLVGRLVSLV
Consensus	(3823)	LVLMVFVTLRSINHD LFSFI LVGRLVSLV

Section 100

	(3862)	3862 3870 3880 3890 3900
avian infectious bronchitis pol 1ab	(3242)	NTLTAYTEGNWELFFELVHTTVLANVSSNSLLGLFVFKC
bovine coronavirus pol 1ab	(3699)	SLWYMGSNLEEELLLMLASLFGTYTWTTALSMAAAKVIA
Human corona 229E pol 1ab	(3409)	AVLYTALYSYDYVSLLVMLLCAISNEWYIGAIIFRICRF
Murine hepatitis pol 1ab	(3786)	SMWYFGANLEEEVLLFLTSLFGTYTWTTMLSLATAKVIA
Consensus	(3862)	SLWY GSNLEEEVLLLLMSLFGTYTWTTILSIA AKVIA

Section 101

	(3901)	3901 3910 3920 3939
avian infectious bronchitis pol 1ab	(3281)	AKWMLYYCNATYLNNYVLMAVMVNCIGWLCTC-EGLYWW
bovine coronavirus pol 1ab	(3738)	KWVAVNVLYFTDIPQIKIVLVCYLFIGYIISCYWGLFSL
Human corona 229E pol 1ab	(3448)	GVAFLPNELVSYFDGVKTVLLFYMLLLEVSCM-YGLLYW
Murine hepatitis pol 1ab	(3825)	KWLAVNVLYFTDVPQIKLVLLSYLCIGYVCCCYWCILSL
Consensus	(3901)	KWLALNVLYFTYIPQIKLVLL YLCIGYVCCCYWGLLSW

Section 102

	(3940)	3940 3950 3960 3978
avian infectious bronchitis pol 1ab	(3320)	VNKVFGLTLCKYNFKVSVDQYRYMCLHKINPPKTVWEVF
bovine coronavirus pol 1ab	(3777)	MNSLFRMPLGV-NYKISVQELRYMNANGLRPPKNSFEAL
Human corona 229E pol 1ab	(3487)	IIRFCKCTLGVYDECV-PAEFKYIVANGLNAPNGPFDAL
Murine hepatitis pol 1ab	(3864)	LNSIFRMPLGVYNYKISVQELRYMNANGLRPPRNSFEAL
Consensus	(3940)	INSIFRMTLGVYNFKISVQELRYMNANGLRPPKNSFEAL

FIGURE 4A (contd.)

		Section 103
	(3979)	3979 3990 4000 4017
avian infectious bronchitis pol 1ab	(3359)	STNILLQGIGGDRVLPIATVQAKLSLVKCTTVVLMQLLT
bovine coronavirus pol 1ab	(3816)	MLNFKLLGIGGVPLIEVSQFCSKLLEVKCANVVILNQLQ
Human corona 229E pol 1ab	(3526)	FLSEKLMGLLGPRTIKVSTVCSKSTTLKCTNVVLMGILS
Murine hepatitis pol 1ab	(3903)	MLNFKLLGIGGVPVIEVSQIQSRLTDVKTANVVLLNCLQ
Consensus	(3979)	MLNFKLLGIGGVRVIEVSTVQSKLTDVKCTNVVLLNCLQ

		Section 104
	(4018)	4018 4030 4040 4056
avian infectious bronchitis pol 1ab	(3398)	KLNVEAHSKMHVVILVEIHKKLASDIVGECMDNILGMLI
bovine coronavirus pol 1ab	(3855)	HLHVASNSKLWQLCSTLHNEILATSDLGVAFEKLAQLLI
Human corona 229E pol 1ab	(3565)	NMNIASSISEWAYCVEMHNKNLCDLPETAQELLLIALA
Murine hepatitis pol 1ab	(3942)	HLHIASNSKLWQYCSTLHNEILATSDLSVAFDKLAQLLV
Consensus	(4018)	HLNIASNSKLWQYCVTLHNKILATSDLGVAFDKLLQLLI

		Section 105
	(4057)	4057 4070 4080 4095
avian infectious bronchitis pol 1ab	(3437)	TLFCIDSTID-------LSEYCDDILKRSIVLQSVTQEFS
bovine coronavirus pol 1ab	(3894)	VLFANPAAVDSKCLTSIEEVCDDYAKDNTVLQALQSEFV
Human corona 229E pol 1ab	(3604)	FFLSKHSDFG-------LGDLVLSYFENDSILCSVASSLV
Murine hepatitis pol 1ab	(3981)	VLFANPAAVDSKCLASIEEVSCDYVRDNTVLCALQSEFV
Consensus	(4057)	VLFANPAAVDSKCL SIEEVCDDYLKDNTVLQALQSEFV

		Section 106
	(4096)	4096 4110 4120 4134
avian infectious bronchitis pol 1ab	(3470)	HTPSYAEYHRLKNLYEKVLVDSKNGGVTQSELAAYRKAA
bovine coronavirus pol 1ab	(3933)	NMASFVEYEVAKKNLDEARSSGSAN---QQQLKQLEKAC
Human corona 229E pol 1ab	(3637)	GMPSFVAYETARQEYENAVANGSS----PDIIKQLKKAM
Murine hepatitis pol 1ab	(4020)	NMASFVEYELAKKNLDEAKASGSAN---QQQIKQLEKAC
Consensus	(4096)	NMPSFVEYELAKKNYDEARASGSAN QQQIKQLEKAC

		Section 107
	(4135)	4135 4140 4150 4160 4173
avian infectious bronchitis pol 1ab	(3509)	NIAKSVFDRDLAVQKKLDSMAERAMTTMYKEARVTDRRA
bovine coronavirus pol 1ab	(3969)	NIAKSAYEEDRAVARKLERMADLALTNMYKEARINDKKS
Human corona 229E pol 1ab	(3672)	NVAKAEFDRESSVQKKINRMAEQAAAMLYKEARAVNRKS
Murine hepatitis pol 1ab	(4056)	NIAKSAYEPDRAVARKLERMADLALTNMYKEARINDKKS
Consensus	(4135)	NIAKSAFDRDRAVQKKLERMADLALTNMYKEARINDKKS

		Section 108
	(4174)	4174 4180 4190 4200 4212
avian infectious bronchitis pol 1ab	(3548)	KLVSSLHALLFSLLKKIDSEKLNVLFDQASSGVVPLATV
bovine coronavirus pol 1ab	(4008)	KVVSALQTMLFSMVRKLDNQALNSILDNAVKGCVPLNAI
Human corona 229E pol 1ab	(3711)	KVVSAMHSLLFGMLRRLDMSSVDTILNMARNGVVPLSVI
Murine hepatitis pol 1ab	(4095)	KVVSALQTMLFSMVRKLDNQALNSILDNAVKGCVPLNAI
Consensus	(4174)	KVVSALQTLLFSMLRKLDNQALNSILDNAVKGVVPLNAI

FIGURE 4A (contd.)

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                                          Section 109
                                 (4213) 4213      4220          4230          4240         4251
avian infectious bronchitis pol 1ab (3587) PIVCSNKFLLVIPPPETNVKCVEGVHCTTSTVVYNIDTV
       bovine coronavirus pol 1ab (4047) PSLAANTLTIIVPDKSVYDQVVDNVYVTYAGNVWQIQTI
     Human corona 229E pol 1ab (3750) PATSAARIVVVVPPHDSFVKMMVDGFVHYAGVVWTLQEV
        Murine hepatitis pol 1ab (4134) PSLTSNTLTLIVPDKQVFDQVVDNVYVTYAGNVWHIQFI
                        Consensus (4213) PSLSANTLTIIVPDKDVFVQVVDNVYVTYAGVVWNIQTI
                                          Section 110
                                 (4252) 4252      4260          4270          4280       4290
avian infectious bronchitis pol 1ab (3626) IDAVCTELHPTSTGSGLTYCISGANIAWPSKVNLTRNGH
       bovine coronavirus pol 1ab (4086) QDSDGTNKQLNEISD---------DCNWPLVIIANRHNE
     Human corona 229E pol 1ab (3789) KDNDGKNVHLKDVTK---------ENQEIDVWPIILTCE
        Murine hepatitis pol 1ab (4173) QDADGAVKQLNEIDV---------NSTWPLVIAANRHNE
                        Consensus (4252) QDADGTNKQLNEIS          N NWPLVI LNRHNE
                                          Section 111
                                 (4291) 4291        4300          4310                  4329
avian infectious bronchitis pol 1ab (3665) NKVDVVLQNNELIVHGVKTKACVAGVDQAHCSVESKCYY
       bovine coronavirus pol 1ab (4116) VSATVLQ-NNELMPAKLKTQVVNS-GPDQTCNTPTQCYY
     Human corona 229E pol 1ab (3819) RVVKIQ--NNELIPEGKMKVKATKG-EGDGGITSEGNAL
        Murine hepatitis pol 1ab (4203) VSTVVLQ-NNELMPQKLRTQVVNS-GSDMNCNTPTQCYY
                        Consensus (4291) VSV VLQ NNELMPAKLKTQVVNS G DA CNTPTQCYY
                                          Section 112
                                 (4330) 4330        4340          4350                  4368
avian infectious bronchitis pol 1ab (3704) TNISGNSVVAAITSSNPNLKVASFLNEAGNQIYVDLDPP
       bovine coronavirus pol 1ab (4153) NNSNNGKIVYAILSDVDGLKYTKILKDDGNFVVLELDPP
     Human corona 229E pol 1ab (3855) NKEGGRAFMYAYVDTKPGMKYVKWEHDSG-VVTVELEPP
        Murine hepatitis pol 1ab (4240) NTGGTGKIVYAILSDCDGLKYTKIVKEDGNCVVLELDPP
                        Consensus (4330) NNSGGGKIVYAILSD PGLKYTKILKDDGN VVLELDPP
                                          Section 113
                                 (4369) 4369         4380          4390                 4407
avian infectious bronchitis pol 1ab (3743) CKFGMKVGVKVEVVYLYFIKNTRSIVRGMVLTATSNVVV
       bovine coronavirus pol 1ab (4192) CKFTVQDVKGLKIKYLYFVKGCNTLARGWVVGTISSTVR
     Human corona 229E pol 1ab (3893) CRFVTDTPTGPQIKYLYFVKNLNNLRRGAVLGYIGATVR
        Murine hepatitis pol 1ab (4279) CKFSVQDVKGLKIKYLYFVKGCNTLARGWVVGTLSSTVR
                        Consensus (4369) CKFSVQDVKGLKIKYLYFVKNCNTLARGWVLGTISSTVR
                                          Section 114
                                 (4408) 4408         4420          4430                 4446
avian infectious bronchitis pol 1ab (3782) LQSKGHETIEEVDAVGILSLCSFAVDPADTYCKYVAAGNQ
       bovine coronavirus pol 1ab (4231) LQAG-TATEYASNSSILSLCAFSVDPKKTYLDFIQQGGT
     Human corona 229E pol 1ab (3932) LQAG-KQTEFVSNSHLLTHCSFAVDPAAAYLDAVKQGAK
        Murine hepatitis pol 1ab (4318) LQAG-TATEYASNSAILSLCAFSVDPKKTYLDYIKQGGV
                        Consensus (4408) LQAG TATEYVSNSAILSLCAFAVDPKKTYLDYIKQGG
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FIGURE 4A (contd.)

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                                                           Section 115
                                  (4447) 4447          4460      4470       4485
avian infectious bronchitis pol 1ab (3821) ILGNLVKMLTVHNGSGFAITSKPSPTPDQDSYGGASVCL
        bovine coronavirus pol 1ab (4269) PIANAVKMLCDHAGTGMAITVKPDATTNQDSYGGASVCI
     Human corona 229E pol 1ab (3970) SVGNCVKMLTNGSGSGQAITCTIDSNTTQDTYGGASVCI
        Murine hepatitis pol 1ab (4356) PVTNCVKMLCDHAGTGMAITIKPEATINQDSYGGASVCI
                         Consensus (4447) PVGNCVKMLTDHAGSGMAITIKPDATTNQDSYGGASVCI

                                                           Section 116
                                  (4486) 4486          4500       4510         4524
avian infectious bronchitis pol 1ab (3860) ICRAHIAHPGSVGNLDGRKQFKGSLPVIETTEKDPVGFC
        bovine coronavirus pol 1ab (4308) YCRARVEHP----DVDGLCKLRGKFVQVPVGIKDPVSYV
     Human corona 229E pol 1ab (4009) YCKAHVAHP----TMDGFCQYKGKWVQVPIGTNDPIRFC
        Murine hepatitis pol 1ab (4395) YCRSRVEHP----DVDGLCKLRGKFVQVPLGIKDPVSYV
                         Consensus (4486) YCRARVEHP    DVDGLCQLKGKFVQVPIGIKDPVSFV

                                                           Section 117
                                  (4525) 4525 4530       4540       4550          4563
avian infectious bronchitis pol 1ab (3899) LRNKVCTVSQCWIGYGCQCDSLRQPKSSVQSVAGASDFD
        bovine coronavirus pol 1ab (4343) LTHDVCQVCGFWRDGSCSCVSTD----------TTVQSKD
     Human corona 229E pol 1ab (4044) LENTVCKVCGCWLNHGCTCDRT-----------ATQSFD
        Murine hepatitis pol 1ab (4430) LTHDVCQVCGFWRDGSCSCVGTG----------SQFQSKD
                         Consensus (4525) LTNDVCQVCGFWRDGSCSCVST           SAIQSKD

                                                           Section 118
                                  (4564) 4564  4570        4580       4590         4602
avian infectious bronchitis pol 1ab (3938) KNYLNRVRGSS-EARLTPLASGCDPDVVKRAFDVCNKES
        bovine coronavirus pol 1ab (4373) TNFLNRVRGTSVDARLVPCASGLSTDVQLRAFDICNASV
     Human corona 229E pol 1ab (4072) NSYLNRVRGS--SAARLEPCNGTDIDYCVRAFDVYNKDA
        Murine hepatitis pol 1ab (4460) TNFLNRVRGTSVNARLVPCASGLDTDVQLRAFDICNANR
                         Consensus (4564) TNFLNRVRGSSVDARLVPCASGLDTDVQLRAFDICNKDA

                                                           Section 119
                                  (4603) 4603   4610       4620        4630        4641
avian infectious bronchitis pol 1ab (3976) AGMFQNLKRNCARFQELRDTEDGNLEYLDSYFVVKQTTP
        bovine coronavirus pol 1ab (4412) AGIGLNLKVNCCRFQRVDENGD----KLDQFFVVKRTDL
     Human corona 229E pol 1ab (4109) SFIGKNDKSNCVKFKNVDKD---------AFYIVKRCIK
        Murine hepatitis pol 1ab (4499) AGIGLYYKVNCCRFQRVDEDGN----KLDKFFVVKRTNL
                         Consensus (4603) AGIGLNLKVNCCRFQRVDEDGD     KLDAFFVVKRT L

                                                           Section 120
                                  (4642) 4642    4650        4660       4670        4680
avian infectious bronchitis pol 1ab (4015) SNYEHEKSCYEDLKS-EVTADHDFFVFNKN---IYNISR
        bovine coronavirus pol 1ab (4447) TIYNPEMECYERVKDCKFVAEHDFFTFDVEGSRVPHIVR
     Human corona 229E pol 1ab (4140) SVMDHEQSMYNLLKGCNAVAKHDFFTWHEGRTIYGNVSR
        Murine hepatitis pol 1ab (4534) EVYNKEKECYELLTHECGVVAEHEFFTFDVEGSRVPHIVR
                         Consensus (4642) SVYNHEKSCYELLKDC VVAEHDFFTFDVEGSRVPNIVR
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FIGURE 4A (contd.)

Section 121

(4681)	4681 4690 4700 4719
avian infectious bronchitis pol 1ab (4050)	QRHTKYTMMDFCYALRHF-PKDCEVLKEILVTYGCIEDY
bovine coronavirus pol 1ab (4486)	KDLTKYTMLDLCYALRHFDRNDCMLLCDIISIYAGCEQS
Human corona 229E pol 1ab (4179)	QDLTKYTMMLLCEALRNFDEKDCEVFKEILVLTGCCSTD
Murine hepatitis pol 1ab (4573)	KDLSKETLDLLDYALRHELRNDCSTDKEILLTYAECEES
Consensus (4681)	QDLTKYTMLDLCYALRHFDRNDCEVLKEILVTYACCEDS

Section 122

(4720)	4720 4730 4740 4758
avian infectious bronchitis pol 1ab (4089)	HPKWFEENFDLYDPIENSKYYVMLAKMGPIVRRALLNAI
bovine coronavirus pol 1ab (4525)	Y----FTKKDWYDFVENPDIINVYKKLGPIFNRALVSAT
Human corona 229E pol 1ab (4218)	Y----FEMKNWFLPI-KEDIHRVYAALGKVVANAMLKCV
Murine hepatitis pol 1ab (4612)	Y----FQKKDWYDFVENPDIINVYKKLGPIFNRALLNTA
Consensus (4720)	Y FEKKDWYDPIENPDIINVYKKLGPIVNRALLNAI

Section 123

(4759)	4759 4770 4780 4797
avian infectious bronchitis pol 1ab (4128)	EFGNLMVEKKLYGVITLDNQDINGKFYDFGDFQKTAPCA
bovine coronavirus pol 1ab (4560)	EFADKLVEVGLVGILTLDNQDLNGKWYDFGDYVIAAPGC
Human corona 229E pol 1ab (4253)	AFCDEMVLKGVVEVLTLDNQDLNDNFYDFGDFVLCPPGM
Murine hepatitis pol 1ab (4647)	KFADALVEACLVHVLTLDNQDLYGQWYDFGDFVKTVPGC
Consensus (4759)	EFAD LVEKGLVGVLTLDNQDLNGKFYDFGDFVKTAPGC

Section 124

(4798)	4798 4810 4820 4836
avian infectious bronchitis pol 1ab (4167)	GVPVFDTYYSYMMPILAMTDAMAPERYFEYD-VHKGYKS
bovine coronavirus pol 1ab (4599)	GVATADSYYSYMMPMLTMCHALDCELYVN-----NAYRL
Human corona 229E pol 1ab (4292)	GLPYCTSTYSYMMPVMGMTNCLASECFMKSDIFGQDFKT
Murine hepatitis pol 1ab (4686)	GVAVADSYYSYMMPMLTMCHALDSELFVN-----GTYRE
Consensus (4798)	GVPVADSYYSYMMPMLTMTHALDSELFVN D NAYKS

Section 125

(4837)	4837 4850 4860 4875
avian infectious bronchitis pol 1ab (4205)	YDLLKYDYIEEKQELFQKYFSYWDQEYHPNCRDCSDDRC
bovine coronavirus pol 1ab (4633)	FDLVQYDFTDYKLELFNKYFKHWSMPYHPNTVDCQDDRC
Human corona 229E pol 1ab (4331)	FDLLKYDFTEHKEVLFNKYFKYWGQDYHPDCVDCHDEMC
Murine hepatitis pol 1ab (4720)	FDLVQYDFTDFKLELFTKYFKHWSMTYHPNTCECEDDRC
Consensus (4837)	FDLLQYDFTDHKLELFNKYFKHWSQDYHPNTVDC DDRC

Section 126

(4876)	4876 4890 4900 4914
avian infectious bronchitis pol 1ab (4244)	LIHCANFNILFSTLLFQTSFCNLCPKVFVLGVPFIATCG
bovine coronavirus pol 1ab (4672)	IIHCANFNILFSMVLPNIGFGPLVRQIFVDGVPFVVSIG
Human corona 229E pol 1ab (4370)	ILHCSNTNTITATTIPNTAFCPLCRKVIIDGVFVVATAG
Murine hepatitis pol 1ab (4759)	IIHCANFNILFSMVLPKICFGPLVRQIFVDGVPFVVSIG
Consensus (4876)	IIHCANFNILFSTVIPNTCFGPLVRQIFVDGVPFVVSIG

FIGURE 4A (contd.)

	Section 127
(4915)	4915 4920 4930 4940 4953
avian infectious bronchitis pol 1ab (4283)	YHSKELGVIMIQPNTMSFSKMGLSQIMQFVGEPALLIGT
bovine coronavirus pol 1ab (4711)	HHYKELGIVMNMDVDTHRYRLSLKDILLYAADPALHVAS
Human corona 229E pol 1ab (4409)	YHFNQIGLVWIKDVNTHSTRLTITELLQFVTEPTLIVAS
Murine hepatitis pol 1ab (4798)	YHYLELGVVMNMDVDTHRYRLSLKDLLLYAADPALHVAS
Consensus (4915)	YHYKELGVVMNMDVDTHRYRLSLKDLLQFVADPALHVAS

	Section 128
(4954)	4954 4960 4970 4980 4992
avian infectious bronchitis pol 1ab (4322)	SNNLVDLELSLSSVCALTSAITHQLILPEHENKDFYDIA
bovine coronavirus pol 1ab (4750)	ASALYDLRTCCFSVAAITSGVKFQTVKPGNFNQDFYDFI
Human corona 229E pol 1ab (4448)	SPALVDLKPIVTLSVAALSTILTSQIVLPGHENKELFDFL
Murine hepatitis pol 1ab (4837)	ASALLDLRTCCFSVAALTSGVKEQTVKPGNFNQDFYEFI
Consensus (4954)	ASALVDLRTCCFSVAAITSGVTFQTVKPGNFNQDFYDFI

	Section 129
(4993)	4993 5000 5010 5020 5031
avian infectious bronchitis pol 1ab (4361)	EKAGMFKEGSSIPDNLHAYPDTGNATLNEYDYYRYNRPT
bovine coronavirus pol 1ab (4789)	LSKGLLKEGSSVDLKHFFFTQDGNAAITDYNYYKYNLPT
Human corona 229E pol 1ab (4487)	RSQSFFDEGSELTLKDFFFTQKSDAAIKEFDYYRYNRPL
Murine hepatitis pol 1ab (4876)	LSKGLLKEGSSVDLKHFFFTQDGNAAITDYNYYKYNLPT
Consensus (4993)	LSKGLLKEGSSVDLKHFFFTQDGNAAITDYNYYKYNRPT

	Section 130
(5032)	5032 5040 5050 5060 5070
avian infectious bronchitis pol 1ab (4400)	MFDLCKLLFCLEVTSKYFECYEGGCIPASQIVINNLDKS
bovine coronavirus pol 1ab (4828)	MVDIKQLLFVLEVVYKYFEIYDGGCIPASQVIVNNYDKS
Human corona 229E pol 1ab (4526)	ALDIGCARVAYQAARYFDCYELGCITTSREVVVTNLNKS
Murine hepatitis pol 1ab (4915)	MVDIKQLLFVLEVVNKYFEIYEGGCIPATQVIVNNYDKS
Consensus (5032)	MVDIKQLLFVLEVVAKYFEIYEGGCIPASQVIVNNYDKS

	Section 131
(5071)	5071 5080 5090 5109
avian infectious bronchitis pol 1ab (4439)	AGYRFNKFGKARLYYEMS-LEEQDQLFEIIKKKVLPTIT
bovine coronavirus pol 1ab (4867)	AGYPFNKFGKARLYYEALSFEEQDEIYAYTKRNVLPTLT
Human corona 229E pol 1ab (4565)	RGWPLLKSGCAGLYYSISYFEQDATFSLEKRNILPTMT
Murine hepatitis pol 1ab (4954)	AGYPFNKFGKARLYYEALSFEEQDEIYAYTKRNVLPTLS
Consensus (5071)	AGYPFNKFGKARLYYEALSFEEQDEIFAYTKRNVLPTLT

	Section 132
(5110)	5110 5120 5130 5148
avian infectious bronchitis pol 1ab (4477)	QMNLKYAISAKNRARIVAGVSILSTMENRQFHQNILKSI
bovine coronavirus pol 1ab (4906)	QMNLKYAISAKNRARTVAGVSILSTMTGRMFHQKCLKSI
Human corona 229E pol 1ab (4604)	QLLLKYAISGFERARTVGGVSLLATMTTRQFHQKCLKSI
Murine hepatitis pol 1ab (4993)	QMNLKYAISAKNRARTVAGVSILSTMTGRMFHQKCLKSI
Consensus (5110)	QMNLKYAISAKNRARTVAGVSILSTMTGRQFHQKCLKSI

FIGURE 4A (contd.)

Section 133

	(5149)	5149 5160 5170 5187
avian infectious bronchitis pol 1ab	(4516)	[illegible]
bovine coronavirus pol 1ab	(4945)	[illegible]
Human corona 229E pol 1ab	(4643)	[illegible]
Murine hepatitis pol 1ab	(5032)	[illegible]
Consensus	(5149)	VATRNVPVVIGTTKFYGGWDNMLRRLIKDVDDPVLMGWD

Section 134

	(5188)	5188 5200 5210 5226
avian infectious bronchitis pol 1ab	(4555)	[illegible]
bovine coronavirus pol 1ab	(4984)	[illegible]
Human corona 229E pol 1ab	(4682)	[illegible]
Murine hepatitis pol 1ab	(5071)	[illegible]
Consensus	(5188)	YPKCDRAMPNILRIVSSLVLARKHDSCCS SDRFYRLAN

Section 135

	(5227)	5227 5240 5250 5265
avian infectious bronchitis pol 1ab	(4594)	[illegible]
bovine coronavirus pol 1ab	(5023)	[illegible]
Human corona 229E pol 1ab	(4721)	[illegible]
Murine hepatitis pol 1ab	(5110)	[illegible]
Consensus	(5227)	ECAQVLSEIVMCGGGYYVKPGGTSSGDATTAFANSVFNI

Section 136

	(5266)	5266 5280 5290 5304
avian infectious bronchitis pol 1ab	(4633)	[illegible]
bovine coronavirus pol 1ab	(5062)	[illegible]
Human corona 229E pol 1ab	(4760)	[illegible]
Murine hepatitis pol 1ab	(5149)	[illegible]
Consensus	(5266)	CQAVSANVCALLSVNG KIEDLSIKALQKRLYSNVYRAD

Section 137

	(5305)	5305 5310 5320 5330 5343
avian infectious bronchitis pol 1ab	(4672)	[illegible]
bovine coronavirus pol 1ab	(5101)	[illegible]
Human corona 229E pol 1ab	(4799)	[illegible]
Murine hepatitis pol 1ab	(5188)	[illegible]
Consensus	(5305)	NVDPAFVSEFYEFLNKHFSMMILSDDGVVCYNSTYASKG

Section 138

	(5344)	5344 5350 5360 5370 5382
avian infectious bronchitis pol 1ab	(4711)	[illegible]
bovine coronavirus pol 1ab	(5140)	[illegible]
Human corona 229E pol 1ab	(4838)	[illegible]
Murine hepatitis pol 1ab	(5227)	[illegible]
Consensus	(5344)	YIANISAFQQVLYYQNNVFMSEAKCWVE DIEKGPHEFC

FIGURE 4A (contd.)

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                                                   Section 139
                                      (5383) 5383   5390      5400      5410       5421
avian infectious bronchitis pol 1ab (4750) SQHTMLVEVDGEPKTLLYPDFSRIIGACVFVLDVDKIEP
        bovine coronavirus pol 1ab (5179) SQHTMLVKMDGDDVYLPYPDPSRILGAGCFVDDLLKTDS
       Human corona 229E pol 1ab (4877) SQHTMQIVDENGKYLDFYTDAHRTISAGVFVDDEITLDA
          Murine hepatitis pol 1ab (5266) SQHTMLVKMDGDEVYLPYPDFSRILGAGCFVDDLLKTDS
                         Consensus (5383) SQHTMLVKMDGDDVYLPYPDPSRILGAGVFVDDLLKTDS

                                                   Section 140
                                      (5422) 5422    5430      5440      5450       5460
avian infectious bronchitis pol 1ab (4789) VAVMEYIATAIEAYPLVHHENEEYKKVFEFVLIAYIRKC
        bovine coronavirus pol 1ab (5218) VLLLERFVSLAIDAYPLVYHENEEYQKVFRVYLEYIKKL
       Human corona 229E pol 1ab (4916) VILLIRYVSLALDAYPLSKHPKPEYRKVFYALLDWVKHL
          Murine hepatitis pol 1ab (5305) VLLIEPFVSLAIDAYPLVYIENPEYQNVFRVYLEYIKKL
                         Consensus (5422) VLLIERFVSLAIDAYPLVYHENPEYQKVFRVYLEYIKKL

                                                   Section 141
                                      (5461) 5461      5470      5480             5499
avian infectious bronchitis pol 1ab (4828) YQELSQNMIMDYSFVMDIDKGSKFWEQEFYENMIRAPTT
        bovine coronavirus pol 1ab (5257) YNDLGNQILDSYAVILSTCDGQKFTDESFYKNMYLRSAV
       Human corona 229E pol 1ab (4955) NKTLNEGVLESFSVTLLDEHESKFWDESFYASMYEKSTV
          Murine hepatitis pol 1ab (5344) YNDLGNQILDSYSVILSTCDGQKFTDETFYKNMYLRSAV
                         Consensus (5461) YNDLGNQILDSYSVILSTCDGSKFWDESFYKNMYLRSTV

                                                   Section 142
                                      (5500) 5500      5510      5520             5538
avian infectious bronchitis pol 1ab (4867) LQSCGVCVVCNSCTILRCGNQTEKPFLCCKCCYDHIMHT
        bovine coronavirus pol 1ab (5296) MQSVGACVVCSSQTSLRCGSCIRKPLLCGKCCYDHVMAT
       Human corona 229E pol 1ab (4994) LQAAGLCVVCGSQTVLRCGDCLRRPMLCTKCAYDHVFGT
          Murine hepatitis pol 1ab (5383) LQSVGACVVCSSQTSLRCGSCIRKPLLCCKCAYDHVMST
                         Consensus (5500) LQSVGACVVCSSQTSLRCGSCIRKPLLCCKCCYDHVMAT

                                                   Section 143
                                      (5539) 5539       5550      5560            5577
avian infectious bronchitis pol 1ab (4906) DHLNVLSINFTITSQLCGGEADVTKLYIGCMSYFCGNHK
        bovine coronavirus pol 1ab (5335) DHKYVLSVSPYVCNAPGCDVNDVTKLYLGGMSYYCEDHK
       Human corona 229E pol 1ab (5033) DHKFILAITPEVCNTSGCNVNDVLKCGLLSLNKYCVDHK
          Murine hepatitis pol 1ab (5422) DHKYVLSVSPYVCNSPGCDVNDVTKLYLGGMSYYCEDHK
                         Consensus (5539) DHKYVLSISPYVCNSPGCDVNDVTKLYLGGMSYYCEDHK

                                                   Section 144
                                      (5578) 5578       5590      5600            5616
avian infectious bronchitis pol 1ab (4945) PKLSIPLVSNGTLGCIYRANCACSENVDDFNQLATTHWS
        bovine coronavirus pol 1ab (5374) PQYSFKLVMNLMVFGLYKQSCTGSPYIDDFNRIASCKWT
       Human corona 229E pol 1ab (5072) PHLSFPLCSAANVFGLYKSSALGSMDIDVFNKLSTSDRS
          Murine hepatitis pol 1ab (5461) PQYSFKLVMNGMVFGLYKQSCTGSPYIEDFNKIASCKWT
                         Consensus (5578) PQYSFPLVSNGMVFGLYKQSCTGSPYIDDFNKIASCKWS
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FIGURE 4A (contd.)

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                                                          Section 145
                                      (5617) 5617        5630      5640        5655
avian infectious bronchitis pol 1ab (4984) IVEPDIHALRCSDSLRRFLAEEVKATDELHKQQFNSAEV
      bovine coronavirus pol 1ab (5413) DVDDYILANECTERLKLEAASTQKATEEAFKQSYASATI
     Human corona 229E pol 1ab (5111) DIRDYKIANDAKESLRLTAAETVKAKEESVKSSYAYATT
      Murine hepatitis pol 1ab (5500) EVDDSVIANECTERLKLFAETTQKATEEAFKQCYASATI
                      Consensus (5617) DVDDYILANECTESLKLFAAETVKATEEAFKQSYASATI

                                                          Section 146
                                      (5656) 5656          5670      5680        5694
avian infectious bronchitis pol 1ab (5023) RTVFSDRELLLLSWEPGKTRPPLIRNYVFTGYHFTRTSKV
      bovine coronavirus pol 1ab (5452) QEIVSERELILSWEIGKVKPPLNKNYVFTGYHFTKNGKT
     Human corona 229E pol 1ab (5150) KEIVGPKELLLLWSGHAKIPLNRNSVFTCFQIMKDSKF
      Murine hepatitis pol 1ab (5539) REIVSDRELILSWEIGKVRPPLNKNYVFTGYHFTNNGKT
                      Consensus (5656) REIVSDRELILSWEIGKVKPPLNKNYVFTGYHFTKNSKT

                                                          Section 147
                                      (5695) 5695 5700         5710      5720      5733
avian infectious bronchitis pol 1ab (5062) QLGDFTFEKGEGKDV-VYYKATSTAKLSVGDIFVLTSHN
      bovine coronavirus pol 1ab (5491) VLGEYVFDKSELTNG-VYYRATTTYKLSVGDVFVLTSHS
     Human corona 229E pol 1ab (5189) QVGEFVFEKVDYGSDTVTYKSTATTKLVPGMLFILTSHN
      Murine hepatitis pol 1ab (5578) VLGEYVFDKSELTNG-VYYRATTTYKLSVGDVFILTSHA
                      Consensus (5695) VLGEFVFDKSELTNG VYYKATTTYKLSVGDVFILTSHN

                                                          Section 148
                                      (5734) 5734   5740       5750      5760      5772
avian infectious bronchitis pol 1ab (5100) VVSLVAPTLCPQQTFSRFVNLRPNVMVPECFVNNIPLYH
      bovine coronavirus pol 1ab (5529) VANLSAPTLVPQE-NYSSIRFASVYSVLETFQNNVVNYQ
     Human corona 229E pol 1ab (5228) VAPLRAPTLMANQEKYSSIYKLHPSFNVSDAYANLVPYYQ
      Murine hepatitis pol 1ab (5616) VSSLSAPTLVPQE-NYTSIRFASVYSVPETFQNNVPNYQ
                      Consensus (5734) VASLSAPTLVPQE NYTSIRLASVYSVPETFQNNVPNYQ

                                                          Section 149
                                      (5773) 5773   5780       5790      5800      5811
avian infectious bronchitis pol 1ab (5139) LVGKQKRTLVQGPPGSGKSHFAIGLAVYFSSARVVFTAC
      bovine coronavirus pol 1ab (5567) HIGMKRYCTVQGPPGTGKSHLAIGLAVYYCTARVVYTAA
     Human corona 229E pol 1ab (5267) LIGKQRITTIQGPPGSGKSHCSIGLGVYYPGARIVFTAC
      Murine hepatitis pol 1ab (5654) HIGMKRYCTVQGPPGTGKSHLAIGLAVYYCTARVVYTAA
                      Consensus (5773) LIGMQRYTTVQGPPGSGKSHLAIGLAVYYCTARVVFTAC

                                                          Section 150
                                      (5812) 5812    5820      5830      5840      5850
avian infectious bronchitis pol 1ab (5178) SHAAVDALCEKAFKFLKVDDCTRIVPQRTTVDCFSKFKA
      bovine coronavirus pol 1ab (5606) SHAAVDALCEKAYKFLNINDCTRIVPAKVRVECYDKFKI
     Human corona 229E pol 1ab (5306) SHAAVDSLCAKAVTAYSVDKCTRIIARARVECYSGFKP
      Murine hepatitis pol 1ab (5693) SHAAVDALCEKAHKFLNINDCTRIVPAKVRVDCYDKFKV
                      Consensus (5812) SHAAVDALCEKAHKFLNINDCTRIVPAKVRVDCYSKFKI
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FIGURE 4A (contd.)

Section 151

	(5851) 5851 5860 5870 5889
avian infectious bronchitis pol 1ab (5217)	NDTGKKYILSTINALLEVSCDILLLNLTSHLTNYEIDFI
bovine coronavirus pol 1ab (5645)	NDTTRKYVFTTINALPEMVTDIVVVDEVSMLTNYELSVI
Human corona 229E pol 1ab (5345)	NNNSAQYVFSTVNALPEVNADIVVVDKVSMCLTYDLSVI
Murine hepatitis pol 1ab (5732)	NDTTRKYVFTTINALPELVTDIIVVDEVSMLTNYELSVI
Consensus (5851)	NDTTRKYVFSTINALPEVVTDIVVVDEVSMLTNYELSVI

Section 152

	(5890) 5890 5900 5910 5928
avian infectious bronchitis pol 1ab (5256)	NGKINYQYVVYVGDPAQLPTLPTHLNG-SLSHKDYLVVL
bovine coronavirus pol 1ab (5684)	NARTRAKHYVYIGDPAQLPAPRVLLSKGTLEPKYFNTVT
Human corona 229E pol 1ab (5384)	NQRISYKHINYVGDPAQLPAPRVLISKGVMEPIDYNVVT
Murine hepatitis pol 1ab (5771)	NSRVSAKHYVYIGDPAQLPAPRVLLNKGTLEPRYFNSVT
Consensus (5890)	NARISYKHYVYIGDPAQLPAPRVLLSKGTLEPKYFNVVT

Section 153

	(5929) 5929 5940 5950 5967
avian infectious bronchitis pol 1ab (5294)	NLMVCVKLDIFLAKCYRCPKEIVDTVSTLVYDGKFIANN
bovine coronavirus pol 1ab (5723)	KLMCCLGPDIFLGTCYRCPKEIVDTVSALVYENKLKAKN
Human corona 229E pol 1ab (5423)	QRMCALGPDVFLHKCQRCPAEIVNTVSELVYENKFVPVK
Murine hepatitis pol 1ab (5810)	KLMCCLGPDIFLGTCYRCPKEIVDTVSALVYNNKLKAKN
Consensus (5929)	KLMCCLGPDIFLGTCYRCPKEIVDTVSALVYENKLKAKN

Section 154

	(5968) 5968 5980 5990 6006
avian infectious bronchitis pol 1ab (5333)	PESRECTYVIVNNGNSDVGHESGSAYITTTLEEVKDFVC
bovine coronavirus pol 1ab (5762)	ESSSLCFKVYYKG---VTTHESSSAVLMQQIYLINKFLK
Human corona 229E pol 1ab (5462)	EASKQLEYLEERG---SVQVDNGSSINRRQLDVVKRFLH
Murine hepatitis pol 1ab (5849)	DNSSMCFKVYYKG---QTTHESSSAVNMQQIHLISKFLK
Consensus (5968)	EASSLCFKVYYKG VTHESSSAVNMQQIHLIKKFLK

Section 155

	(6007) 6007 6020 6030 6045
avian infectious bronchitis pol 1ab (5372)	RNKQCREYILSPYNAMIQRAYHMILKNVSLTDSSQGSE
bovine coronavirus pol 1ab (5798)	ANPLWHKAVFISPYNSQNFAAKRVLGIQTQTVDSAQGSE
Human corona 229E pol 1ab (5498)	KNSTWSKAVFISPYNSQNYVAKRLLGLQTQTVDSAQGSE
Murine hepatitis pol 1ab (5885)	ANPSWSNAVFISPYNSQNYVAKRVLGLQTQTVDSAQGSE
Consensus (6007)	ANPSWSKAVFISPYNSQNYVAKRVLGLQTQTVDSAQGSE

Section 156

	(6046) 6046 6060 6070 6084
avian infectious bronchitis pol 1ab (5411)	NLYVIFCVIADSQHALNINRFNVALTRAKRGILVVMRQR
bovine coronavirus pol 1ab (5837)	YDYVIYSQTAETAHSVNVNRFNVAITRAKKGILCVMSNM
Human corona 229E pol 1ab (5537)	YDYVTFAQTSDTAHACNANRFNVAITRAKKGIFCIMSDR
Murine hepatitis pol 1ab (5924)	YDYVIYSQTAETAHSVNVNRFNVAITRAKKGILCVMSSM
Consensus (6046)	YDYVIFSQTADTAHAVNVNRFNVAITRAKKGILCVMSNR

FIGURE 4A (contd.)

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                                                                                Section 157
                                (6085) 6085  6090          6100          6110          6123
avian infectious bronchitis pol 1ab (5450) DELYSALKFTELDSETS-------LQGTGEFHILNKEFS
     bovine coronavirus pol 1ab (5876) QLFEALQFTTLTLDKVPQAVETRVQCSTNLFKDCSKSYS
    Human corona 229E pol 1ab (5576) TLEDALKFFEITMTDLQ-----SESS-CGLFKDIARNPI
        Murine hepatitis pol 1ab (5963) QLFESLNFTTLTLDKHN---NPRLQCTTNLFLDCSRSYV
                       Consensus (6085) QLFEALNFTTLTLDKIN     RLQCSTNLFKDCSKSYS
                                                                                Section 158
                                (6124) 6124   6130          6140          6150          6162
avian infectious bronchitis pol 1ab (5482) GVHPAYAVTTKALAATYKVNDELLALVNVEAGSEILKH
     bovine coronavirus pol 1ab (5915) GYHPAHAPSFLAVDDKYLATGDLAVCLGIGDS-AVTYSR
    Human corona 229E pol 1ab (5609) DLPPSHATTYLSLSDRFKTSGDLAVQIGNNN--VCLYEH
        Murine hepatitis pol 1ab (5999) GYHPAHAPSFLAVDDKYKVGGDLAVCLNVADS-AVTYSR
                       Consensus (6124) GYHPAHAPSFLALDDKYKVSGDLAVCLNVADS AVTYSR
                                                                                Section 159
                                (6163) 6163    6170          6180          6190          6201
avian infectious bronchitis pol 1ab (5521) LISLLSFKMSVNVEGCHNMFITTRDEALRNVPGAVGFDV
     bovine coronavirus pol 1ab (5953) LISLMGFKLDVTLDLYCKLSLTKEEAVKRVPAWVGFDAE
    Human corona 229E pol 1ab (5646) VIEYMGHRFDVSMPGSHSLICLRDFAMRHVKGILGMDVS
        Murine hepatitis pol 1ab (6037) LISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAE
                       Consensus (6163) LISLMGFKLDVTLDGYHKLFITRDEAIKRVRAWVGFDVE
                                                                                Section 160
                                (6202) 6202    6210          6220          6230          6240
avian infectious bronchitis pol 1ab (5560) ATIACGTNLSTNLPFFQVCFSTGALDLVTPEGLVDTSIGN
     bovine coronavirus pol 1ab (5992) GAHATRDSTGTHFPLQLGFSTGIDFVVEATGLFADRDGY
    Human corona 229E pol 1ab (5685) GAHVLGDNVGTNVPLQVGFESNGVEFVAQPEGCVLTNTGS
        Murine hepatitis pol 1ab (6076) GAHAIRDSIGTHFPLQLGFSTGIDFVVEATGMFAERDGY
                       Consensus (6202) GAHATRDSIGTNFPLQLGFSTGIDFVVEPTGLVATRDGY
                                                                                Section 161
                                (6241) 6241     6250          6260                        6279
avian infectious bronchitis pol 1ab (5599) NFEPVNSKAPPGEQFDNLLRVLFKSAKPWHVTRPRIVQML
     bovine coronavirus pol 1ab (6031) SFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQMF
    Human corona 229E pol 1ab (5724) VVKPVRARAPPGEQFTHLVPLLRKGQPWSVLRKRIVQMI
        Murine hepatitis pol 1ab (6115) VFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIPRIVQML
                       Consensus (6241) VFKPV AKAPPGEQFKHLIPLMSRGQPWDVVRPRIVQML
                                                                                Section 162
                                (6280) 6280     6290          6300                        6318
avian infectious bronchitis pol 1ab (5638) ADNLCNVSDCVVFVTWCHGLELTTLRYFVKIG-KEQVCS
     bovine coronavirus pol 1ab (6070) ADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNV
    Human corona 229E pol 1ab (5763) ADFLAGSSDVVLVFVLWAGGLELTTMRYFVKIG-AVKHCQ
        Murine hepatitis pol 1ab (6154) SDHLADLADSVVLVTWAASFELTCLRYFAKVGREVVCSV
                       Consensus (6280) ADHLADLSDCVVLVTWAAGLELTTLRYFVKIGREV CCV
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FIGURE 4A (contd.)

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                                                                       Section 163
                                  (6319) 6319       6330        6340            6357
 avian infectious bronchitis pol 1ab (5676) CGSRLITFHSHTQAYAGWKHCLGFDFVYNPLLVDIQQWG
         bovine coronavirus pol 1ab (6109) CTKRAIAYHERTGYYGCWRHSVTGDYLYNPLIVDIQQWG
        Human corona 229E pol 1ab (5801) CGTVAICYHSVSNDYCCFKHALGCDYVYNLYVIDIQQWG
           Murine hepatitis pol 1ab (6193) CTKRATCFNSRTGYYGCWRHSYSCDYLYNPLIVDIQQWG
                        Consensus (6319) CTKRATCFNSRTGYYGCWKHSLGCDYLYNPLIVDIQQWG

                                                                       Section 164
                                  (6358) 6358         6370        6380          6396
 avian infectious bronchitis pol 1ab (5715) YSGNLQFSSDLHCNVHGHAHVASVDAIMPHCLAINNATQ
         bovine coronavirus pol 1ab (6148) YITSLSSNHDIYCSVHKGAHVASSDAIMTRCLAVYDCFC
        Human corona 229E pol 1ab (5840) YVGSLSTSHAICNVHRNEHVASGDAIMTRCLAVYDCFV
           Murine hepatitis pol 1ab (6232) YTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFC
                        Consensus (6358) YTGSLSSNHDLICSVHKGAHVASSDAIMTRCLAVYDCFC

                                                                       Section 165
                                  (6397) 6397          6410        6420         6435
 avian infectious bronchitis pol 1ab (5754) QDVNWDLTYPHIANLDEVNSSCRYLQRMYLNACVDALKV
         bovine coronavirus pol 1ab (6187) NNINWNVEYPIISNLLSINTSGRVLQRVMLKAAMLCNRY
        Human corona 229E pol 1ab (5879) KNVDWSITYPMIANENAINKGGRTVQSHIMRAAIKLYNP
           Murine hepatitis pol 1ab (6271) KSVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRY
                        Consensus (6397) KNVNWNLTYPIIANELSINTSCRLLQRVMLRAAMLCNRY

                                                                       Section 166
                                  (6436) 6436           6450        6460        6474
 avian infectious bronchitis pol 1ab (5793) NVVYDIGNPKGI-KCVRRGDVNFRFYDKNPIVRNVKQFE
         bovine coronavirus pol 1ab (6226) TVCYDIGNPK---AIACVKDFDFKFYDAQPIVKSVKTLL
        Human corona 229E pol 1ab (5918) KAIHDIGNPK--GIRCAVTDAKWYCYDKNPINSNVKTLE
           Murine hepatitis pol 1ab (6310) DVCYDIGNPK---GLACVKGYDFKFYDASPVVKSVKQFV
                        Consensus (6436)  VCYDIGNPK    AIACVKDFDFKFYDKNPIVKSVKTLE

                                                                       Section 167
                                  (6475) 6475  6480        6490        6500        6513
 avian infectious bronchitis pol 1ab (5831) YDYNQHKDKFAFDGLCMFWNCNVLCYDNSLVPCYVDSNL
         bovine coronavirus pol 1ab (6262) YSFEAHKDSFKFDGLCMFWNCNVYLKYPNAVVCRFDTRVL
        Human corona 229E pol 1ab (5955) YDYMTHG--QMFDGLCLLWNCNVDMYPEFSIVCRFDLHTR
           Murine hepatitis pol 1ab (6346) YTYEAHKDQFLFDGLCMFWNCNVDKYPANAVVCRFDTRVL
                        Consensus (6475) YDYEAHKD FLDGLCMFWNCNVDKYPDNAVVCRFDTRVL

                                                                       Section 168
                                  (6514) 6514  6520        6530        6540        6552
 avian infectious bronchitis pol 1ab (5870) SVFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFF
         bovine coronavirus pol 1ab (6301) NNLNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFF
        Human corona 229E pol 1ab (5992) STLNLEGCVNGSLYVNNHATHTPAYDKRAMAKLPAPFF
           Murine hepatitis pol 1ab (6385) NKLNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFF
                        Consensus (6514) S LNLPGCNGGSLYVNKHAFHTPPFDRAAFENLKPMPFF
```

FIGURE 4A (contd.)

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                                                            Section 169
                                   (6553) 6553   6560      6570      6580      6591
  avian infectious bronchitis pol 1ab (5909) FYDSSPFETTQVDGVAQ-DLVSLATKDCITKCNIGGAVC
        bovine coronavirus pol 1ab (6340) Y-SDTPCVYMDGMDAKQVDYVPLKSATCIIRCNIGGAVC
        Human corona 229E pol 1ab (6031) YSDDGSCEVVHDQVN----YVPIRATNCIIKCNIGGAVC
       Murine hepatitis pol 1ab (6424) Y-SDTPCVYMEGMESKQVDYVPLRSATCITRCNLGGAVC
                       Consensus (6553) YYSDTPCVYMDGMDAKQVDYVPLRSATCITKCNIGGAVC
                                                            Section 170
                                   (6592) 6592   6600      6610      6620      6630
  avian infectious bronchitis pol 1ab (5947) KHHAQMYAEEVTSYTAAVTAGFTFWTNKLNPYNLWKSF
        bovine coronavirus pol 1ab (6379) LKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTF
        Human corona 229E pol 1ab (6066) SKHANLYRAAVEEYNIFTQAGFNIWVPTTFDCYNLWQTF
       Murine hepatitis pol 1ab (6463) LKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTF
                       Consensus (6592) LKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTF
                                                            Section 171
                                   (6631) 6631    6640      6650                  6669
  avian infectious bronchitis pol 1ab (5986) SAL--QSTDNIAYNMYKGGHYDAIAGEMPTVITGDKVFV
        bovine coronavirus pol 1ab (6418) TKL--QSLENIVYNLVKTGHYTGQAGEMPCAIINDKVVA
        Human corona 229E pol 1ab (6105) ---LQGLENIAFNVVNKGSFVGADGELPVAISGDKVFV
       Murine hepatitis pol 1ab (6502) ----QSLENVVYNLVNAGHFDGRAGELPCAVIGDKVIA
                       Consensus (6631) TKL  QSLENIVYNLVNAGHFDG AGELPCAIIGDKVFV
                                                            Section 172
                                   (6670) 6670      6680      6690               6708
  avian infectious bronchitis pol 1ab (6023) IDQGVEKAVFVNQTTLPTSVAFELYAKRNIRTLPNNRIL
        bovine coronavirus pol 1ab (6455) KIDKEDVVVFKNNTTYPTNVAVELFAKRSIRHHPELKIF
        Human corona 229E pol 1ab (6144) RDGNTDNLVFVNKTSLPTNIAFELFAKRKVGLTPPLSIL
       Murine hepatitis pol 1ab (6539) KIQNEDVVVFKNNTTFPTNVAVELFAKRSIRPHPELKIL
                       Consensus (6670) KIQNEDVVVFVNNTTLPTNVAVELFAKRSIR HPELKIL
                                                            Section 173
                                   (6709) 6709       6720      6730              6747
  avian infectious bronchitis pol 1ab (6062) KGLGVDVTNGFVIWDYANQTPLYRNTVKVCAYT--DIEP
        bovine coronavirus pol 1ab (6494) RNLNIDVCWKHVIWDYARESIFCSNTYGVCMYTDLKFID
        Human corona 229E pol 1ab (6183) KNLGVVATYKFVLWDYEAERPLTSFTKSVCGYT--DFAE
       Murine hepatitis pol 1ab (6578) RNLNIDVCWSHVLWDYAKDSVFCSSTYKVCKYTDLQCIE
                       Consensus (6709) KNLNIDVTWKHVIWDYAKESPLCSNTYKVCAYTDLDFIE
                                                            Section 174
                                   (6748) 6748        6760      6770               6786
  avian infectious bronchitis pol 1ab (6099) NGLVVLYDDRYNDYQSFLAADNAVLVSTQCYKRYSYVEI
        bovine coronavirus pol 1ab (6533) KLNVLFDGRDNGALEAFKRSNNGVYISTTKVKSLSMIKG
        Human corona 229E pol 1ab (6220) DVCTCYDNSIQGSYERFTLSTNAVLFSATAVKTGGKS--
       Murine hepatitis pol 1ab (6617) SLNVLFDGRDNGALEAFKKCRNGVYINTTKIKSLSMIKG
                       Consensus (6748)  LNVLFDGRDNGAYEAFKKS NAVYISTTKVKSLSMIKG
```

FIGURE 4A (contd.)

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                                                                         Section 175
                                    (6787) 6787         6800       6810          6825
avian infectious bronchitis pol 1ab (6138) PSNLLVQNGMPLKDGAN-------------LYVYKRVN-
       bovine coronavirus pol 1ab (6572) PPRAEDNGVVVDKVGDTDCVFYFAVRKEGQDVIFSQFDS
        Human corona 229E pol 1ab (6257) LPAIKLNFGMINGNAIATVKSEDGNIKNINWFVYVRKDG
          Murine hepatitis pol 1ab (6656) PQRADLNGVVVEKVGDSDVEFWFAVRKDGDDVIFSRTGS
                          Consensus (6787) PPRADLNGVMVDKVGDSDV FWFAVRKDGNDVIFSR DS

                                                                         Section 176
                                    (6826) 6826          6840       6850          6864
avian infectious bronchitis pol 1ab (6163) -----------------------GAFVTLPNTTNQGSSY
       bovine coronavirus pol 1ab (6611) LRVSSNQSPQGNLGSNEPGNVGGNDALATSTIFTQSRVL
        Human corona 229E pol 1ab (6296) KPVDHYDG------------------------FYIIGRNT
          Murine hepatitis pol 1ab (6695) LEPSHYRSPQGNPGGNRVGDLSGNEALARGTIFTQSRLL
                          Consensus (6826) L VSHY SPQGN G N  G L GNDALA  TIFTQSRLL

                                                                         Section 177
                                    (6865) 6865  6870       6880       6890          6903
avian infectious bronchitis pol 1ab (6180) ELTEPRSDIERLLLAMSEESFVERLG-KLLGLQHLLGE
       bovine coronavirus pol 1ab (6650) SSFTCRTDMEKDFLALDQDDFIQKYGLEDYAFEHIVYGN
        Human corona 229E pol 1ab (6312) QDFLPTSTMEEDLLNMDIGVFIQKYGLEDFNFEHIVVGD
          Murine hepatitis pol 1ab (6734) SSFTPRSEMEKDLMDLDDDVFIAKYSLQDYAFEHVVYGS
                          Consensus (6865) SSFTPRSDMEKDFLALDDDVFIQKYGLEDYAFEHIVYGD

                                                                         Section 178
                                    (6904) 6904  6910       6920       6930          6942
avian infectious bronchitis pol 1ab (6218) VDKPQLGGLHTVIGMYKLLRANKLNAKSVTNSDSDVMQN
       bovine coronavirus pol 1ab (6689) FNQKIIGGLHLLIGLYTRQQTSNLVIQEFVSYDSSIHSY
        Human corona 229E pol 1ab (6351) VSKTTLQGLHLLISQVFLSKMGILKAEEFVAASDITLKC
          Murine hepatitis pol 1ab (6773) FNQKIIGGLHLLIGLAFRQQKSNLVIQEFVTYDSSIHSY
                          Consensus (6904) VNQKIIGGLHLLIGLYRRQQ SNLVIQEFVSYDSSIHSY

                                                                         Section 179
                                    (6943) 6943  6950       6960       6970          6981
avian infectious bronchitis pol 1ab (6257) YFVLSDNG-SYKQLCTVVLLLLDDFIELLRNLLKEYGTN
       bovine coronavirus pol 1ab (6728) FITDEKSG-GSKSVCIVIDLLLDDFVALVKSLNLNCVS-
        Human corona 229E pol 1ab (6390) CTVTYLNDPSSKTVCIYMSLLLDDFVSVLKSLDLTVVS-
          Murine hepatitis pol 1ab (6812) FITDENSG-SSKSVCTVIDLLLDDFVDIVKSLNLKCVS-
                          Consensus (6943) FIVDE SG SSKSVCTVIDLLLDDFVELLKSLNL CVS

                                                                         Section 180
                                    (6982) 6982   6990       7000       7010          7020
avian infectious bronchitis pol 1ab (6295) KSIYVTVSIDYHSINFMTVFEDGSIKTCTPQLQS--AMT
       bovine coronavirus pol 1ab (6765) --KVVNVNVDFKDFQFMLWQNDEKVMTFYPRLQAASDWK
        Human corona 229E pol 1ab (6428) --KVHEVIIFNKPWRWMLLCKDNAVATFYPQLQS-AEWK
          Murine hepatitis pol 1ab (6849) --KVVNVNVDFKDFQFMLWCNEEKVMTFYLRLQAAADWK
                          Consensus (6982)   KVVNVNIDFKDFQFMLWCNDEKVMTFYPRLQAAADWK
```

FIGURE 4A (contd.)

Section 181

(7021)	7021 7030 7040 7059
avian infectious bronchitis pol 1ab (6332)	CFSNIPEDYKVQNCVMSPCNIPHLCVGITDFSSILMNVA
bovine coronavirus pol 1ab (6802)	PGYSLSVLYKYLNSPMERVSLWNYGKPVTLPTGCMMNVA
Human corona 229E pol 1ab (6464)	CYLSMIGLKKTQRMCLSPCNLYNIGAGLKLPSGIMFNVV
Murine hepatitis pol 1ab (6886)	PGYVMPVLYKYLESPLERVNLWNYGKPITLPTGCMMNVA
Consensus (7021)	PGYSMPVLYKYQNSPLERVNLWNYGKPITLPSGIMMNVA

Section 182

(7060)	7060 7070 7080 7098
avian infectious bronchitis pol 1ab (6371)	KYTQLCQYLSKPLICVPHTTFVMHFGAGSDKGVAPGSTA
bovine coronavirus pol 1ab (6841)	KYVQLCQYLNTTTLAVPHVMRVLHLGAGSEKGVAPGSAV
Human corona 229E pol 1ab (6503)	KITQLCQAFNSTILCVPHRMHVLHLGAGNDYGVAPGSTA
Murine hepatitis pol 1ab (6925)	KYTQLCQYLSTTILAVPANMRVLHLGAGSDKGVAPGSAV
Consensus (7060)	KYTQLCQYLSTTTLCVPHNMRVLHLGAGSDKGVAPGSAV

Section 183

(7099)	7099 7110 7120 7137
avian infectious bronchitis pol 1ab (6410)	LKQWLPEGTLLVDNDIVDYVSFAHVSVLSDDNKYNTEHK
bovine coronavirus pol 1ab (6880)	LRQWLPAGTILVDNDLYPFVSDSVATYFGDCITLPFDCQ
Human corona 229E pol 1ab (6542)	LKRWLEHDAIVTIDHDVVDYVSADFSVTGDCATVYLEDK
Murine hepatitis pol 1ab (6964)	LRQWLPAGSILVDNDVNPFLSDSVASYYGNCITLPFDCQ
Consensus (7099)	LKQWLPAGTILVDNDVVPFVSDAVASYFGDCITLPFDCQ

Section 184

(7138)	7138 7150 7160 7176
avian infectious bronchitis pol 1ab (6449)	FDLVSDLLYTDNDSKRKHEGLIANNGNDDVFIYLSSFLR
bovine coronavirus pol 1ab (6919)	WDIITSDMLDPFTKNIGEYNVSK----DGFFTYICHMIR
Human corona 229E pol 1ab (6581)	FDLLTSDLYDGRIKAIDGENVSK----EGFFTYLNGFIC
Murine hepatitis pol 1ab (7003)	WDLITSDLYDPLTKNIGEYNVSK----DGFFTYLCHLIR
Consensus (7138)	FDLIISDMYDPITKNIGEYNVSK DGFFTYICHFIR

Section 185

(7177)	7177 7190 7200 7215
avian infectious bronchitis pol 1ab (6488)	NNLALGGFPVKVVELTSKHEVLYDIAQDCAWSTMFCSAV
bovine coronavirus pol 1ab (6954)	DKLALGGSVALLITEFSWNAELYKLMGYFAFWTNFCTNA
Human corona 229E pol 1ab (6616)	EKLAIGGSIFIKVLEYSWNKKLYELVQRFSFWLMFETSV
Murine hepatitis pol 1ab (7038)	DKLALGGSVAIINTEFSWNAELYSLMGKFAFWTILCINV
Consensus (7177)	DKLALGGSVAIKITEFSWNAELYDLMQKFAFWTMFCTNV

Section 186

(7216)	7216 7230 7240 7254
avian infectious bronchitis pol 1ab (6527)	NASSSLASLITVNYLG-ASEKVKVSCKTLHANYIIWPNC
bovine coronavirus pol 1ab (6993)	NAISSEGFLIGINYLG--KPKVEIDGNVMHANYLFWRNS
Human corona 229E pol 1ab (6655)	NTSSSLAFVVGLIYLGDFAQGPFIDGNIIHPIIVFWRNS
Murine hepatitis pol 1ab (7077)	NASSSEGFLIGINWLN--KTRTEIDGKTMHANYLFWRNS
Consensus (7216)	NASSSEAFLIGINYLG K KVEIDGNTMHANYLFWRNS

FIGURE 4A (contd.)

```
                                                                 Section 187
                                  (7255) 7255 7260      7270      7280     7293
avian infectious bronchitis pol 1ab (6565) NYLQTSAYLIFLVAPLDLRLKATPLVNEKTEQKTDLLFN
        bovine coronavirus pol 1ab (7030) TVWNGGAYSLFDMAKFPLKLAGTAVINLRADQINDMVYS
       Human corona 229E pol 1ab (6694) TVMSLSYNSVLLLSKFNCKHKAIVVVQLKDSDINEMFLS
         Murine hepatitis pol 1ab (7114) TMWNGGAYSLFDMSKFPLKAAGTAVVSLKPDQINDLVLS
                        Consensus (7255) TVWNGSAYSLFDMAKFPLKLKATAVVNLK DQINDLVLS
                                                                 Section 188
                                  (7294) 7294  7300              7319
avian infectious bronchitis pol 1ab (6604) DIKC--LLVRDVGNTSFTSDSFVCTM SEQ ID NO: 9905
        bovine coronavirus pol 1ab (7069) ILEKGNILVRDTNKEVFVGDSLVNVI SEQ ID NO: 9886
       Human corona 229E pol 1ab (6733) IVRSF-LLVRGNGKCLSFSNHLVSTK SEQ ID NO: 9914
         Murine hepatitis pol 1ab (7153) TIEKGTILVRDTRKEVFVGDSLVNVK SEQ ID NO: 9887
                        Consensus (7294) LIEKGKLLVRDTGKEVFVSDSLVNVK
```

FIGURE 4B

```
                                                                     Section 1
                                    (1) 1        ,10       20                39
         human coronavirus OC43 NP  (1) MSFTPGKQSSS-RASSGNRSGNGILK---WADQSDQVRN
                  Bovine corona NP  (1) MSFTPGKQSSS-RASFGNRSGNGILK---NADQSDQSRN
avian infectious bronchitis virus NP  (1) -------------------MASGKAAG--KSDSP--AP-
           mouse hepatitis virus NP  (1) MSFVPGQENAGGRSSSVNRAGNGILKKTTWADQTERGPN
                         Consensus  (1) MSFTPGKQSSS RASSGNRSGNGILK   WADQSDQARN
                                                                     Section 2
                                   (40) 40       50        60                78
         human coronavirus OC43 NP (36) VQTRGRRAQPKQTATSQQPSGGNVVPYYSWFSGITQFQK
                  Bovine corona NP (36) VQTRGRRAQPKQTATSQLPSGGNVVPYYSWFSGITQFQK
avian infectious bronchitis virus NP (16) VIKLGGPKPPKVGSSGN----------ASWFQALKAKKL
           mouse hepatitis virus NP (40) NQNRGRRNQPKQTATTQ-PNSGSVVPHYSWFSGITQFQK
                         Consensus (40) VQTRGRRAQPKQTATSQ PSGGNVVPYYSWFSGITQFQK
                                                                     Section 3
                                   (79) 79          ,90       ,100          117
         human coronavirus OC43 NP (75) GKEFEFVEGQGVPIAPGVPATEAKGYWYRHNRRSFKTAD
                  Bovine corona NP (75) GKEFEFAEGQGVPIAPGVPATEAKGYWYRHNRRSFKTAD
avian infectious bronchitis virus NP (45) NAPAPKFEGSGVPDNENLKNSQQHGYWRRQAR--FKPGK
           mouse hepatitis virus NP (78) GKEFQFAEGQGVPIANGIPASEQKGYWYRHNRRSFKTPD
                         Consensus (79) GKEFEFAEGQGVPIAPGVPASEQKGYWYRHNRRSFKTAD
                                                                     Section 4
                                  (118) 118         ,130       ,140          156
         human coronavirus OC43 NP (114) GNQRQLLPRWYFYYLGTGPHAKDQYGTDIDGVYWVASNQ
                  Bovine corona NP (114) GNQRQLLPRWYFYYLGTGPHAKDQYGTDIDGVFWVASNQ
avian infectious bronchitis virus NP  (82) GGRKPVPDAWYFYYTGTGPAADLNWGDSQDGIVWVAAKG
           mouse hepatitis virus NP (117) GQQKQLLPRWYFYYLGTGPHAGASYGDSIEGVFWVANSQ
                         Consensus (118) GNQKQLLPRWYFYYLGTGPHAKDQYGTSIDGVFWVASNQ
                                                                     Section 5
                                  (157) 157         ,170       ,180          195
         human coronavirus OC43 NP (153) ADVNTPADIVDRDPSSDEAIPTRFPPG---TVLPQGYYI
                  Bovine corona NP (153) ADVNTPADILDRDPSSDEAIPTRFPPG---TVLPQGYYI
avian infectious bronchitis virus NP (121) ADVKSRSNQGTRDPDKFDQYPLRFSDGGPDGNFRWDFIP
           mouse hepatitis virus NP (156) APTNTRSDIVEHDPSSHEAIPTRFAPG---TVLPQGFYV
                         Consensus (157) ADVNTRADIVDRDPSSDEAIPTRFPPG   TVLPQGFYI
                                                                     Section 6
                                  (196) 196           210        220         234
         human coronavirus OC43 NP (189) EGSGRSAPNSRSTSRTSSRASSAGSRSRANSGNRTPTSG
                  Bovine corona NP (189) EGSGRSAPNSRSTSRASSRASSAGSRSRANSGNRTPTSG
avian infectious bronchitis virus NP (160) LSRGRSGRSTAASSAASSRVPSR-------EGSRGRRSG
           mouse hepatitis virus NP (192) EGSGRSAPASRSGSRSQSRGPNN--RARSSSNQRQPAST
                         Consensus (196) EGSGRSAPNSRSTSRASSRASSAGSRSRANSGNRTPTSG
```

FIGURE 4B (contd.)

Section 7

	(235)	235 240 250 260 273
human coronavirus OC43 NP	(228)	VTPDMADQIASLVLAKLGKDATKPQQVTKHTAKEVRQKI
Bovine corona NP	(228)	VTPDMADQIASLVLAKLGKDATKPQQVTKQTAKEIRQKI
avian infectious bronchitis virus NP	(192)	AEDSLIARAAKIIQDQQ----RKGTRITKQKAEEMAHRR
mouse hepatitis virus NP	(229)	VKPLMAEEIAALVLAKLGKDAGQPKQVLKQSAKEVRQKI
Consensus	(235)	VTPDMADQIASLVLAKLGKDATKPQQVTKQTAKEVRQKI

Section 8

	(274)	274 280 290 300 312
human coronavirus OC43 NP	(267)	LNKPRQKRSPNKQCTVQQCFGKTGPNQ--NFGGGEMLKL
Bovine corona NP	(267)	LNKPRQKRSPNKQCTVQQCFGKLGPNQ--NFGGGEMLKL
avian infectious bronchitis virus NP	(227)	FCK----KTVPPGYRIDQVFGPRTKGKEGNFGDDKMNEE
mouse hepatitis virus NP	(268)	LNKPRQKSTPNKQCPVQQCFGKRGPNQ--NFGGSEMLKL
Consensus	(274)	LNKPRQKRSPNKQCTVQQCFGKRGPNQ NFGGGEMLKL

Section 9

	(313)	313 320 330 340 351
human coronavirus OC43 NP	(304)	GTSDPQFPILAELAPTAGAFFFGSRLELAKVQN------
Bovine corona NP	(304)	GTSDPQFPILAELAPTAGAFFFGSRLELAKVQN------
avian infectious bronchitis virus NP	(262)	GIKDGRVTAMLNLTFSPHACLGCSRVTPKLQPDGLHLRF
mouse hepatitis virus NP	(305)	GTSDPQFPILAELAPTVGAFFFGSKLELVKKN-------
Consensus	(313)	GTSDPQFPILAELAPTAGAFFFGSRLELAKVQN

Section 10

	(352)	352 360 370 380 390
human coronavirus OC43 NP	(337)	---LSGNPDEPQKDVYELRYNGAIRFDSTLSGFETIMKV
Bovine corona NP	(337)	---LSGNLDEPQKDVYELRYNGAIRFDSTLSGFETIMKV
avian infectious bronchitis virus NP	(301)	EFTTVVPRDDTQFDNYVKICDECVDGVGTRPKDEVVRPK
mouse hepatitis virus NP	(337)	----SGGADEPTKDVYELQYSGAVRFDSTLPGFETIMKV
Consensus	(352)	LSGN DEPQKDVYELRYNGAIRFDSTLSGFETIMKV

Section 11

	(391)	391 400 410 429
human coronavirus OC43 NP	(373)	LNENLNAYQQQDGMMNMSPKPQSQRGHKNGQ---GENDN
Bovine corona NP	(373)	LNENLNAYQQQDGMMNMSTKPQQQRGQKNGQ---GENTN
avian infectious bronchitis virus NP	(340)	SRPNS---RPATRTSSPAPRQPRQKKEKKSK---KQDDE
mouse hepatitis virus NP	(372)	LNEHLNAYQKDGGADVVSPKPQI-KGRRQAQEKKDEVDN
Consensus	(391)	LNENLNAYQQQDGMMNMSPKPQRQKG KNGQ GENDN

Section 12

	(430)	430 440 450 468
human coronavirus OC43 NP	(409)	ISVAVPKSRVQQNKSRELTAEDISLLKKMDEP-----YT
Bovine corona NP	(409)	ISVAAPKSRVQQNKSRELTAEDISLLKKMDEP-----YT
avian infectious bronchitis virus NP	(373)	VDKALTSDEERNN--AQIEFDDEPKVINWGDS------A
mouse hepatitis virus NP	(410)	VSVPKPKSSVQRIVSRELTPEDRSLLAQILDDGVVPDGL
Consensus	(430)	ISVALPKSRVQQNKSRELTAEDISLLKKMDDP YT

Section 13

	(469)	469 474	
human coronavirus OC43 NP	(443)	EDTSEI	SEQ ID NO: 9915
Bovine corona NP	(443)	EDTSEI	SEQ ID NO: 9887
avian infectious bronchitis virus NP	(404)	LGENEL	SEQ ID NO: 9906
mouse hepatitis virus NP	(449)	EDDSNV	SEQ ID NO: 9898
Consensus	(469)	EDTSEI	

FIGURE 4C

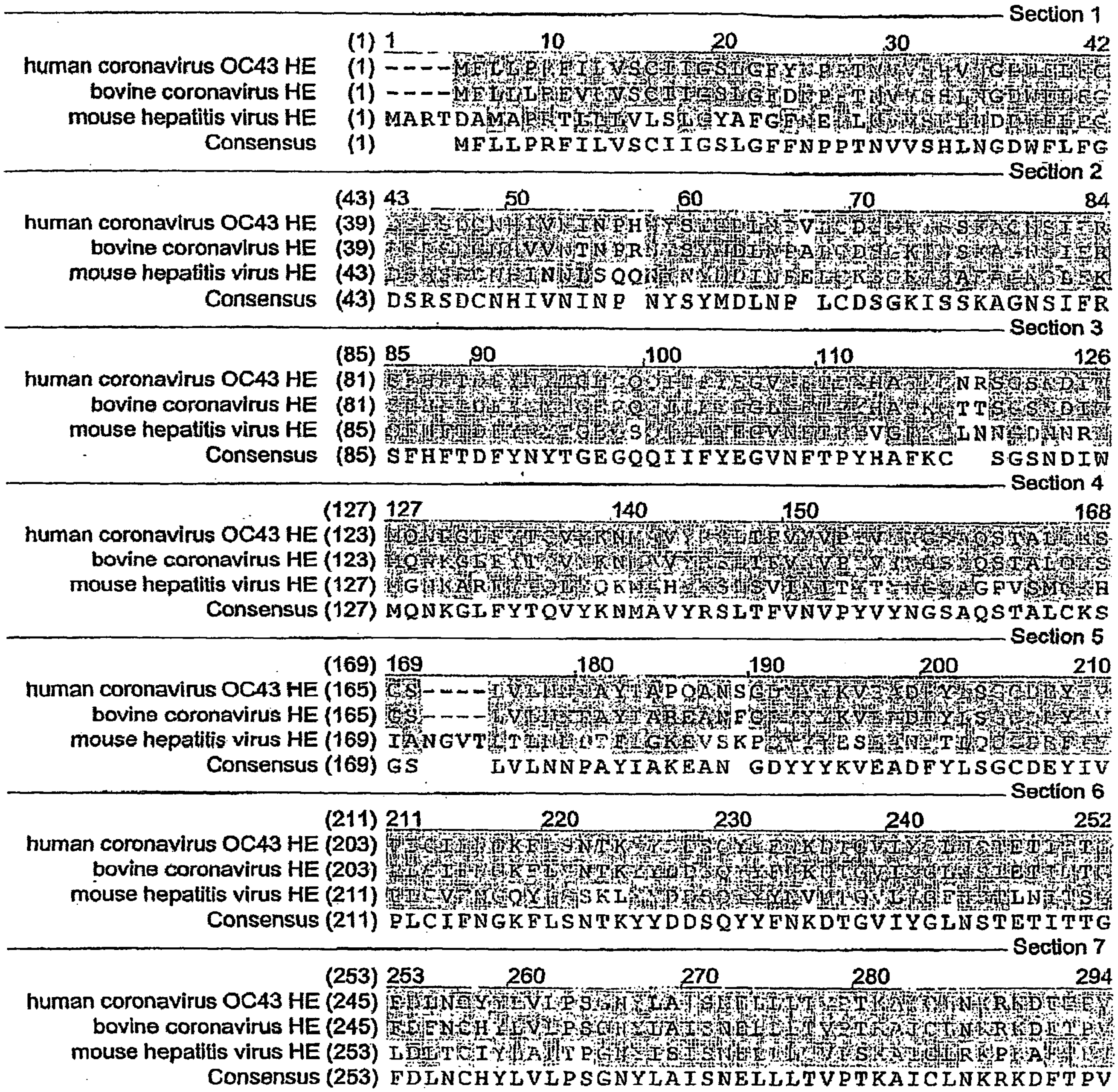
Section 1
(1) 1 10 20 30 42
human coronavirus OC43 HE (1)
bovine coronavirus HE (1)
mouse hepatitis virus HE (1)
Consensus (1) MFLLPRFILVSCIIGSLGFFNPPTNVVSHLNGDWFLFG
Section 2
(43) 43 50 60 70 84
human coronavirus OC43 HE (39)
bovine coronavirus HE (39)
mouse hepatitis virus HE (43)
Consensus (43) DSRSDCNHIVNINP NYSYMDLNP LCDSGKISSKAGNSIFR
Section 3
(85) 85 90 100 110 126
human coronavirus OC43 HE (81)
bovine coronavirus HE (81)
mouse hepatitis virus HE (85)
Consensus (85) SFHFTDFYNYTGEGQQIIFYEGVNFTPYHAFKC SGSNDIW
Section 4
(127) 127 140 150 168
human coronavirus OC43 HE (123)
bovine coronavirus HE (123)
mouse hepatitis virus HE (127)
Consensus (127) MQNKGLFYTQVYKNMAVYRSLTFVNVPYVYNGSAQSTALCKS
Section 5
(169) 169 180 190 200 210
human coronavirus OC43 HE (165)
bovine coronavirus HE (165)
mouse hepatitis virus HE (169)
Consensus (169) GS LVLNNPAYIAKEAN GDYYYKVEADFYLSGCDEYIV
Section 6
(211) 211 220 230 240 252
human coronavirus OC43 HE (203)
bovine coronavirus HE (203)
mouse hepatitis virus HE (211)
Consensus (211) PLCIFNGKFLSNTKYYDDSQYYFNKDTGVIYGLNSTETITTG
Section 7
(253) 253 260 270 280 294
human coronavirus OC43 HE (245)
bovine coronavirus HE (245)
mouse hepatitis virus HE (253)
Consensus (253) FDLNCHYLVLPSGNYLAISNELLLTVPTKAICLNKRKDFTPV

FIGURE 4C (contd.)

Section 8

```
                                   295    300         310         320             336
                          (295)
human coronavirus OC43 HE (287)    QVVDSRWNNARQSDNMLAVACQPPYCYFRNSTTNYVGVYDIN
     bovine coronavirus HE (287)   QVVDSRWNNARQSDNMLAVACQPPYCYFRNSTTNYVGVYDIN
  mouse hepatitis virus HE (295)   QVVDDSWHSNGSSDNMLAIGCQLPYCYFRHTTSDYNGVYDSH
                 Consensus (295)   QVVDSRWNNARQSDNMTAVACQPPYCYFRNSTTNYVGVYDIN
```

Section 9

```
                                   337          350        360                378
                          (337)
human coronavirus OC43 HE (329)    HGDAGFTSILSGLLYNSPCFSQQGVFRYDNVSSVWPLYPYGR
     bovine coronavirus HE (329)   HGDAGFTSILSGLLYDSPCFSQQGVFRYDNVSSVWPLYSYGR
  mouse hepatitis virus HE (337)   HGDAGFTSILAGLMYNVSCLAQQGAFVYNNVSSSWPQYPYGH
                 Consensus (337)   HGDAGFTSILSGLLYNSPCFSQQGVFRYDNVSSVWPLYPYGR
```

Section 10

```
                                   379        390        400        410        420
                          (379)
human coronavirus OC43 HE (371)    CPTAADINNPDLPICVYDPLPVILLGILLGVAVIIIVVLLLY
     bovine coronavirus HE (371)   CPTAADINTPDVPICVYDPLPIILLGILLGVAVIIIVVLLLY
  mouse hepatitis virus HE (379)   CPTAANIVF-MAPVCMYDPLPVILLGVLLGIAVLIIVFLMFY
                 Consensus (379)   CPTAADIN PDLPICVYDPLPVILLGILLGVAVIIIVVLLLY
```

Section 11

```
                                   421        432
                          (421)
human coronavirus OC43 HE (413)    FMVDNGTRLHDA    SEQ ID NO: 9916
     bovine coronavirus HE (413)   FMVDNGTRLHDA    SEQ ID NO: 9888
  mouse hepatitis virus HE (420)   FMTESSVRLLEA    SEQ ID NO: 9899
                 Consensus (421)   FMVDNGTRLHDA
```

FIGURE 4D

Section 1

```
                                          (1)  1        10        20                 39
                     bovine coronavirus Sm (1)  MFMADAYFADTVWYVGQIIFIVAICLLVII--VVVAFLA
avian infectious bronchitis virus Sm      (1)  MNNLLNKSLEENGSFLTALYIIVGFLALYLLGRALQAFVQ
               mouse hepatitis virus Sm   (1)  --MFNLFLTDTVWYVGQIIFLFAVCLMVTI--IVVAFLA
                              Consensus   (1)  M M N FL DTVWYVGQIIFIVAICLLVII  IVVAFLA
```

Section 2

```
                                          (40)  40        50        60               78
                     bovine coronavirus Sm (38)  TFKLCIQLCGMCNTLVLSPSIYVFNRGRQFYEFYN-DVK
avian infectious bronchitis virus Sm      (40)  AADALCLFWYTWVVIPGAKGTAFVYKYTYGRKLNNPELE
               mouse hepatitis virus Sm   (36)  SIKLCIQLCGLCNTLVLSPSIYLYDRSKQLYKYYNEEMR
                              Consensus   (40)  S KLCIQLCGLCNTLVLSPSIYLF R KQ YKFYN ELK
```

Section 3

```
                                          (79)  79       90                  108
                     bovine coronavirus Sm (76)  PPVLDVDDV---------------------  SEQ ID NO: 9889
avian infectious bronchitis virus Sm      (79)  AVIVNEFPKNGWNNKNPANFQDAQRDKLYS  SEQ ID NO: 9907
               mouse hepatitis virus Sm   (75)  LPLLEVDDI---------------------  SEQ ID NO: 9900
                              Consensus   (79)   PILDVDDI
```

FIGURE 4E

```
                                                                  Section 1
                                     (1) 1        10        20        30       40
         human coronavirus OC43 M    (1) -MSSKTTPAPVYIWTADEAIKFLKEWNFSLGIILLFITII
             bovine coronavirus M    (1) -MSSVTTPAPVYTWTADEAIKFLKEWNFSLGIILLFITII
avian infectious bronchitis virus M  (1) -----MSNAANCTLDCEQSVELFKEYNLFITAFLLFLTII
           mouse hepatitis virus M   (1) MTSTTQAPQPVYQWTADEAIRFLKEWNFSLGIILLFVTII
                         Consensus   (1)  MSS TTPAPVYTWTADEAIKFLKEWNFSLGIILLFITII
                                                                  Section 2
                                    (41) 41       50        60        70       80
         human coronavirus OC43 M   (40) LQFGYTSRSMFVYVIKMIILWLMWPLTIILTIFNCVYALN
             bovine coronavirus M   (40) LQFGYTSRSMFVYVIKMIILWLMWPLTIILTIFNCVYALN
avian infectious bronchitis virus M (36) LQYGYATRSRFIYIMKMIVLWCFWPLNIAVGVISCIYPPN
           mouse hepatitis virus M  (41) LQFGYTSRSMFVYVVKMIILWLMWPLTIVLCIFNCVYALN
                         Consensus  (41) LQFGYTSRSMFVYVIKMIILWLMWPLTIILTIFNCVYALN
                                                                  Section 3
                                    (81) 81       90        100       110     120
         human coronavirus OC43 M   (80) NVYLGLSIVFTIVAIIMWIVYFVNSIRLFIRTGSWWSFNP
             bovine coronavirus M   (80) NVYLGFSIVFTIVAIIMWIVYFVNSIRLFIRTGSWWSFNP
avian infectious bronchitis virus M (76) TGGIVAAKILLHVVACLSFEGYWIQKYIKKFGSQWYAFIH
           mouse hepatitis virus M  (81) NVYLGFSIVFTIVSIIMWIMYFVNSIRLFIRTGSWWSFNP
                         Consensus  (81) NVYLGFSIVFTIVAIIMWIVYFVNSIRLFIRTGSWWSFNP
                                                                  Section 4
                                   (121) 121      130       140       150     160
         human coronavirus OC43 M  (120) ETNNLMCIDMKGTMYVRPIIEDYHTLTVTIIRGHLYIQGI
             bovine coronavirus M  (120) ETNNLMCIDMKGRMYVRPIIEDYHTLTVTIIRGHLYMQGI
avian infectious bronchitis virus M (116) KSNAVGSILLSNGQQCNFAIESVPMVLSPIIKNGVLYCEG
           mouse hepatitis virus M (121) ETNNLMCIDMKGTVYVRPIIEDYHTLTATIIRGHLYMQGV
                         Consensus (121) ETNNLMCIDMKGTMYVRPIIEDYHTLTVTIIRGHLYMQGI
                                                                  Section 5
                                   (161) 161      170       180       190     200
         human coronavirus OC43 M  (160) KLGTGYSWADLPAYMTVAKVTHLCTYKRGFLDRISDTSGF
             bovine coronavirus M  (160) KLGTGYSLSDLPAYVTVAKVSHLLTYKRGFLDKIGDTSGF
avian infectious bronchitis virus M (156) QWLAKCEPDHLPKDIFVCTPDRRNIYR-----------MV
           mouse hepatitis virus M (161) KLGTGFSLSDLPAYVTVAKVSHLCTYKRAFLDKVDGVSGF
                         Consensus (161) KLGTGYSLSDLPAYVTVAKVSHLCTYKRGFLDKI DTSGF
                                                                  Section 6
                                   (201) 201      210       220       231
         human coronavirus OC43 M  (200) AVYVKSKVGNYRLPSTQKGSGMDTALLRNNI  SEQ ID NO: 9917
             bovine coronavirus M  (200) AVYVKSKVGNYRLPSTQKGSGMDTALLRNNI  SEQ ID NO: 9890
avian infectious bronchitis virus M (185) QKYTGDQSGNKKRFAT---------------  SEQ ID NO: 9908
           mouse hepatitis virus M (201) AVYVKSKVGNYRLPSN-KPSGMDTALLRI--  SEQ ID NO: 9901
                         Consensus (201) AVYVKSKVGNYRLPSTQKGSGMDTALLRNNI
```

FIGURE 4F

```
                                                                                Section 1
                                     (1) 1                  10                  20                  30       40
       human coronavirus OC43 S      (1) MFLILLISLPTAFAVIGDLKCTSDN-INDKDTGPPPISTD
 avianinfectiousbronchitisvirusS     (1) ----------------------------------------
           bovine coronavirus S      (1) MFLILLISLPTAFAVIGDLKCTTVS-INDVDTGVPSISTD
         mouse hepatitis virus S     (1) MLFVFILFLPSCLGYIGDFRCIQLVNSNGANVSAPSISTE
                       Consensus     (1) MFLILLISLPTAFAVIGDLKCTSL  IND DTG PSISTD
                                                                                Section 2
                                    (41) 41                 50                  60                  70       80
       human coronavirus OC43 S     (40) TVDVTNGLGTYYVLDRVYLNTTLFLNGYYPTSGSTYRNMA
 avianinfectiousbronchitisvirusS     (1) ------------------MLVTPLLLVTLLCALCSAVLYD
           bovine coronavirus S     (40) TVDVTNGLGTYYVLDRVYLNTTLLLNGYYPTSGSTYRNMA
         mouse hepatitis virus S    (41) TVEVSQGLGTYYVLDRVYLNATLLLTGYYPVDGSKFRNLA
                       Consensus    (41) TVDVTNGLGTYYVLDRVYLNTTLLLNGYYPTSGSTYRNMA
                                                                                Section 3
                                    (81) 81                 90                 100                 110      120
       human coronavirus OC43 S     (80) LKGSVLLSRLWFKPPFLSDFINGIFAKVKNTKVIKDRVMY
 avianinfectiousbronchitisvirusS    (23) SSSYVYYYQSAFRPPSGWHLQGGAYAVVNISSEFNNAGSS
           bovine coronavirus S     (80) LKGTLLLSTLWFKPPFLSDFTNGIFAKVKNTKVIKDGVMY
         mouse hepatitis virus S    (81) LTGTNSVSLSWFQPPYLSQFNDGIFAKVQNLKTSTPSGAT
                       Consensus    (81) LKGTVLLS SWFKPPFLSDFNNGIFAKVKNTKVIKDAVMY
                                                                                Section 4
                                   (121) 121                130                 140                 150      160
       human coronavirus OC43 S    (120) SEFPAITIGSTFVNTSYSVVVQPRTINSTQDGDNKLQGLL
 avianinfectiousbronchitisvirusS    (63) SGCTVGITHGGRVVNASSIAMTAP--------S-------
           bovine coronavirus S    (120) SEFPAITIGSTFVNTSYSVVVQP----HTTILGNKLQGFL
         mouse hepatitis virus S   (121) AYFPTIVIGSLFGYTSYTVVIEP-------YNG-----VI
                       Consensus   (121) SEFPAITIGSTFVNTSYSVVVQP       T   GNKLQGLL
                                                                                Section 5
                                   (161) 161                170                 180                 190      200
       human coronavirus OC43 S    (160) EVSVCQYNMCEYPQTICHPNLG-NHRKELWHLDTGVVSCL
 avianinfectiousbronchitisvirusS    (88) --SGMAWSSSQFCTAHCNFSDTTVFVTHCYKHGGCPLTGM
           bovine coronavirus S    (156) EISVCQYTMCEYPNTICNPNLG-NQRVELWHWDTGVVSCL
         mouse hepatitis virus S   (149) MASVCQYTICLLPYTDCKPNTNGNKLIGFWHTDVKPPICV
                       Consensus   (161) EISVCQYTMCEYPNTICNPNLG N RIELWH DTGVVSCL
                                                                                Section 6
                                   (201) 201                210                 220                 230      240
       human coronavirus OC43 S    (199) YKRNFTYDVNADYLYFHFYQEGGTFYAYFTDTGVVTKFLF
 avianinfectiousbronchitisvirusS   (126) LQQNLIRVSAMKNGQLFYNLTVSVAKYPTFRSFQCVNNLT
           bovine coronavirus S    (195) YKRNFTYDVNADYLYFHFYQEGGTFYAYFTDTGVVTKFLF
         mouse hepatitis virus S   (189) LKRNFTLNVNADAFYFHFYQHGGTFYAYYADKPSATTFLF
                       Consensus   (201) YKRNFTYDVNADYLYFHFYQEGGTFYAYFTDTGVVTKFLF
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FIGURE 4F (contd.)

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                                                                               Section 7
                                   (241) 241      250        260        270       280
          human coronavirus OC43 S (239) NVYLGMAISHYYVMPLTCN----SKLTLEYWVTPLTSRQY
 avianinfectiousbronchitisvirusS (166) SVYLNGDIVYTSNETIDVTSAGVYFKAGGPITYKVMREVK
               bovine coronavirus S (235) NVYLGTVLSHYYVMPLTCN----SALTLEYWVTPLTSKQY
            mouse hepatitis virus S (229) SVYLGDILTQYYVLPFICNPTAGSTFAPRYWVTPLVKRQY
                          Consensus (241) SVYLG ILSHYYVMPLTCN  A S LTLEYWVTPLTSRQY
                                                                               Section 8
                                   (281) 281      290        300        310       320
          human coronavirus OC43 S (275) LLAFNQDGIIFNAEDCMSDFMSEIKCKTQSIAPPTGVYEL
 avianinfectiousbronchitisvirusS (206) ALAYFVNGTAQDVILCDGSPRGLLACQYNTGNFSDGFYPF
               bovine coronavirus S (271) LLAFNQDGVIFNAVDCKSDFMSEIKCKTLSIAPSTGVYEL
            mouse hepatitis virus S (269) LFNFNQKGVITSAVDCASSYTSEIKCKTQSMLPSTGVYEL
                          Consensus (281) LLAFNQDGVIFNAVDC SSFMSEIKCKTQSIAPSTGVYEL
                                                                               Section 9
                                   (321) 321      330        340        350       360
          human coronavirus OC43 S (315) NGYTVQPIADVYRKKPNLPNCNIEAWLNDKSVPSPLNWER
 avianinfectiousbronchitisvirusS (246) TNSSLVKQKFIVYK---------ENSVNTTCTLHNFIEHN
               bovine coronavirus S (311) NGYTVQPIADVYRRIPNLPDCNIEAWLNDKSVPSPLNWER
            mouse hepatitis virus S (309) SGYTVQPVGVVYRRVANLPACNIEEWLTARSVPSPLNWER
                          Consensus (321) NGYTVQPIADVYRRIPNLP CNIEAWLNDKSVPSPLNWER
                                                                              Section 10
                                   (361) 361      370        380        390       400
          human coronavirus OC43 S (355) KTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFSSI
 avianinfectiousbronchitisvirusS (277) ETGAIPNP------SGVANIQTYQTKTAQSGYYNFNFSFL
               bovine coronavirus S (351) KTFSNCNFNMSSLMSFIQAYSFTCNNIDAAKIYGMCFSSI
            mouse hepatitis virus S (349) KTFQSCNFNLSSLLRYVQAESLFCNNIDASKVYGRCFGSI
                          Consensus (361) KTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFSSI
                                                                              Section 11
                                   (401) 401      410        420        430       440
          human coronavirus OC43 S (395) TIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLY
 avianinfectiousbronchitisvirusS (311) SSFVYKESNFMYGSYHPSCKFRLETINNGLWFNSLSVSIA
               bovine coronavirus S (391) TIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLY
            mouse hepatitis virus S (389) SVDKFAVPRSRQVDLQLGNSGFLQTANYKIDTAATSCQLH
                          Consensus (401) SIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLY
                                                                              Section 12
                                   (441) 441      450        460        470       480
          human coronavirus OC43 S (435) YNLPAANVSVSRFNPSTWNKRFGFIEDSVFKPRPAGVLTN
 avianinfectiousbronchitisvirusS (351) LGP-----------------------------LQG-----
               bovine coronavirus S (431) YNLPAANVSVSRFNPSTWNRRFGFTEQSVFKPQPAGVFTD
            mouse hepatitis virus S (429) YTLPKNNVTINNHNPSSWNRRYGFNDAGVFGKN------Q
                          Consensus (441) YNLPAANVSVSRFNPSTWNRRFGF E SVFKPNPAGV TN
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FIGURE 4F (contd.)

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                                                              Section 13
                                 (481) 481      490       500       510       520
      human coronavirus OC43 S   (475) HDVVYAQHCFKAPKNFCPCKLNG-SCVGSGP-----GKNN
avianinfectiousbronchitisvirusS  (357) ---GCKQSVFKGRATCCYAYSYGGPSLCKGVYSG------
          bovine coronavirus S   (471) HDVVYAQHCFKASTNFCPCKLDGSLCVGNGPGIDAGYKTS
        mouse hepatitis virus S  (463) HDVVYAQCCFTVRSSYCPCAQPDIVSPCTTQTK------P
                      Consensus  (481) HDVVYAQHCFKARSNFCPCKL G LSVGSGP      K
                                                              Section 14
                                 (521) 521      530       540       550       560
      human coronavirus OC43 S   (509) GIGTCPAGTNYLTCDN------LCTPDPIT--FTGTYKCP
avianinfectiousbronchitisvirusS  (388) ----------------------------------------
          bovine coronavirus S   (511) GIGTCPAGTNYLTCHNAAQCDCLCTPDPITSKATGPYKCP
        mouse hepatitis virus S  (497) ----------------------------------------
                      Consensus  (521) GIGTCPAGTNYLTC N      LCTPDPIT   TG YKCP
                                                              Section 15
                                 (561) 561      570       580       590       600
      human coronavirus OC43 S   (541) QTKSLVGIGEHCSGLAVKSDYCG----GNSCTCRPQAFLG
avianinfectiousbronchitisvirusS  (388) ----ELDHNFECGLIVYVTKSGG-----------------
          bovine coronavirus S   (551) QTKYLVGIGEHCSGLAIKSDHCG----GNPCTCQPQAFLG
        mouse hepatitis virus S  (497) -KSAFVNVGDHCEGLGVLEDNCGNADPHKGCICANNSFIG
                      Consensus  (561) QTKALVGIGEHCSGLAVKSDHCG    GN CTC PQAFLG
                                                              Section 16
                                 (601) 601      610       620       630       640
      human coronavirus OC43 S   (577) WSADSCLQGDKCNIFANFILHDVNSGLTCSTDLQKANTDI
avianinfectiousbronchitisvirusS  (407) -----------------SR-IQTATEPPVITQNNYNNI
          bovine coronavirus S   (587) WSVDSCLQGDRCNIFANFILHDVNSGTTCSTDLQKSNTDI
        mouse hepatitis virus S  (536) WSHDTCLVNDRCQIFANILLNGINSGTTCSTDLQLPNTEV
                      Consensus  (601) WS DSCLQGDRCNIFANFILHDINSGTTCSTDLQKANTDI
                                                              Section 17
                                 (641) 641      650       660       670       680
      human coronavirus OC43 S   (617) ILGVCVNYDLYGILGQGIFVEVNATYYNSWQNLLYDSNGN
avianinfectiousbronchitisvirusS  (427) TDNTQRDYNFIYRTEQSFITNNTDSAVS--YNYLADNGLA
          bovine coronavirus S   (627) ILGVCVNYDLYGITGQGIFVEVNATYYNSWQNLLYDSNGN
        mouse hepatitis virus S  (576) VTGICVKYDLYGITGQGVFKEVKADYYNSWQTLLYDVNGN
                      Consensus  (641) ILGVCVNYDLYGITGQGIFVEVNATYYNSWQNLLYDSNGN
                                                              Section 18
                                 (681) 681      690       700       710       720
      human coronavirus OC43 S   (657) LYGFRDYIINRTFMIRSCYSGRVSAAFHANSSEPALLFRN
avianinfectiousbronchitisvirusS  (465) ILDTSGSIDIFVVQGEYGLNYYKVNPCEDVNQQFVVSGGK
          bovine coronavirus S   (667) LYGFRDYLTNRTFMIRSCYSGRVSAAFHANSSEPALLFRN
        mouse hepatitis virus S  (616) LNGFRDLTTNKTYTIRSCYSGRVSAAFHKDAPEPALLYRN
                      Consensus  (681) LYGFRDYITNRTFMIRSCYSGRVSAAFHANSSEPALLFRN
```

FIGURE 4F (contd.)

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                                                                    Section 19
                                   (721) 721      730       740       750       760
       human coronavirus OC43 S    (697) IKCNYVFNNSLTRQLQPINYFDSYLGCVVNAYNSTAISVQ
avianinfectiousbronchitisvirusS    (505) LVGILTSRNETGSQLLENQFYIKITNGTRRFRRSITENVA
            bovine coronavirus S   (707) IKCNYVFNNTLSRQLQPINYFDSYLGCVVNADNSTSSVVQ
        mouse hepatitis virus S    (656) INGSMVFSHNISREENPLNYFDSYLGCVVNADNRTDEALP
                      Consensus    (721) IKCNYVFNNSLSRQLQPINYFDSYLGCVVNADNSTSEAVQ
                                                                    Section 20
                                   (761) 761      770       780       790       800
       human coronavirus OC43 S    (737) TCDLTVGSGYCVDYSKNRRSRGAITTGYRFTNFEPFTVNS
avianinfectiousbronchitisvirusS    (545) NCPYVSYGKFCIKPDG--------------SIATIVPKQL
            bovine coronavirus S   (747) TCDLTVGSCYLVDYSTKRRSRRSITTGYRFTNFEPFTVNS
        mouse hepatitis virus S    (696) NGDIRMGAGLCVDYSKSRRADRSVSTGYRLTTFEPYTPML
                      Consensus    (761) TCDLTVGSGYCVDYSK RRSRRSITTGYRFTNFEPFTVNS
                                                                    Section 21
                                   (801) 801      810       820       830       840
       human coronavirus OC43 S    (777) VNDSLEPVGGLYEIQIPSEFTIGNMVEFIQTSSPKVTIDC
avianinfectiousbronchitisvirusS    (571) EQFVAPLFNVTENVLIPNSFNLTVTDEYIQTRMDKVQINC
            bovine coronavirus S   (787) VNDSLEPVGGLYEIQIPSEFTIGNMEEFIQTSSPKVTIDC
        mouse hepatitis virus S    (736) VNDSVQSMDGLYEMQIPTNFTIGHHEEFIQTRSPKVTIDC
                      Consensus    (801) VNDSLEPVGGLYEIQIPSEFTIGNMEEFIQTSSPKVTIDC
                                                                    Section 22
                                   (841) 841      850       860       870       880
       human coronavirus OC43 S    (817) AAFVCGDYAACKSQLVEYGSFCDNINAILTEVNELLDTTQ
avianinfectiousbronchitisvirusS    (611) LQYVCGSSLDCRKLFQQYGPVCDNILSVVNSVGQKEDMEL
            bovine coronavirus S   (827) SAFVCGDYAACKSQLVEYGSFCDNINAILTEVNELLDTTQ
        mouse hepatitis virus S    (776) AAFVCGDNTACRQQLVEYGSFCVNVNAILNEVNNLLDNMQ
                      Consensus    (841) AAFVCGDYAACKSQLVEYGSFCDNINAILTEVNELLDTTQ
                                                                    Section 23
                                   (881) 881      890       900       910       920
       human coronavirus OC43 S    (857) LQVANSLMNGVTLSTKLKDGVNFNVDDINFSPVLGCLGSE
avianinfectiousbronchitisvirusS    (651) LNFYSSTKP-----AGFNTPVLSNVSTGEFNISLLLTNPS
            bovine coronavirus S   (867) LQVANSLMNGVTLSTKLKDGVNFNVDDINFSPVLGCLGSD
        mouse hepatitis virus S    (816) LQVASALMQGVTISSRLPDGISGPIDDINFSPLLGCIGST
                      Consensus    (881) LQVASSLMNGVTLSTKLKDGVNFNVDDINFSPVLGCLGSS
                                                                    Section 24
                                   (921) 921      930       940       950       960
       human coronavirus OC43 S    (897) CSKASS-------RSAIEDLLFDKVKLSDVGFVEAYNNCT
avianinfectiousbronchitisvirusS    (686) SRRKRS---------LIEDLLFTSVESVGLPTNDAYKNCT
            bovine coronavirus S   (907) CNKVSS-------RSAIEDLLFSKVKLSDVGFVEAYNNCT
        mouse hepatitis virus S    (856) CAEDGNGPSAIRGRSAIEDLLFDKVKLSDVGFVEAYNNCT
                      Consensus    (921) CAK SS       RSAIEDLLFDKVKLSDVGFVEAYNNCT
```

FIGURE 4F (contd.)

Section 25

	(961)	961 970 980 990 1000
human coronavirus OC43 S	(930)	GGAE--IRDLICVQSYKGIKVLPPLLSENQISGYTLAATS
avianinfectiousbronchitisvirusS	(717)	ACPLGFFKDLACAREYNGLLVLPPIITAEMQALYTSSLVA
bovine coronavirus S	(940)	GGAE--IRDLICVQSYNGIKVLPPLLSENQISGYTLAATS
mouse hepatitis virus S	(896)	GGQE--VRDLLCVQSFNGIKVLPPVLSESQISGYTTGATA
Consensus	(961)	GGAE IRDLICVQSYNGIKVLPPLLSENQISGYTLAATA

Section 26

	(1001)	1001 1010 1020 1030 1040
human coronavirus OC43 S	(968)	ASLFPPWTAAAGVPFYLNVQYRINGLGVTMDVLSQNQKLI
avianinfectiousbronchitisvirusS	(757)	SMAFGGITAAGAIPFATQLQARINHLGITQSLLLKNQEKI
bovine coronavirus S	(978)	ASLFPPWSAAAGVPFYLNVQYRINGLGVTMDVLSQNQKLI
mouse hepatitis virus S	(934)	AAMFPPWSAAAGVPFSLSVQYRINGLGVTMNVLSENQKMI
Consensus	(1001)	ASLFPPWSAAAGVPFYLNVQYRINGLGVTMDVLSQNQKLI

Section 27

	(1041)	1041 1050 1060 1070 1080
human coronavirus OC43 S	(1008)	ANAFNNALYAIQEGFDATNSALVKIQAVVNANAEALNNLL
avianinfectiousbronchitisvirusS	(797)	AASFNKAIGHMQEGFRSTSLALQQIQDVVSKQSAILTETM
bovine coronavirus S	(1018)	ANAFNNALGAIQEGFDATNSALVKIQAVVNANAEALNNLL
mouse hepatitis virus S	(974)	ASAFNNALGAIQDGFDATNSALGKIQSVVNANAEALNNLL
Consensus	(1041)	ANAFNNALGAIQEGFDATNSALVKIQAVVNANAEALNNLL

Section 28

	(1081)	1081 1090 1100 1110 1120
human coronavirus OC43 S	(1048)	QQLSNRFGAISASLQEILSRLDALEAEAQIDRLINGRLTA
avianinfectiousbronchitisvirusS	(837)	ASLNKNFGAISSVIQEIYQQFDAIQANAQVDRLITGRLSS
bovine coronavirus S	(1058)	QQLSNRFGAISSSLQEILSRLDALEAQAQIDRLINGRLTA
mouse hepatitis virus S	(1014)	NQLSNRFSAISASLQEILTRLEAVEAKAQIDRLINGRLTA
Consensus	(1081)	QQLSNRFGAISASLQEILSRLDALEANAQIDRLINGRLTA

Section 29

	(1121)	1121 1130 1140 1150 1160
human coronavirus OC43 S	(1088)	LNAYVSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFC
avianinfectiousbronchitisvirusS	(877)	LSVLASAKQAEYIRVSQQRELATQKINECVKSQSIRYSFC
bovine coronavirus S	(1098)	LNAYVSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFC
mouse hepatitis virus S	(1054)	LNAYIAKQLSDSTLIKVSAAQAIEKVNECVKSQTTRINFC
Consensus	(1121)	LNAYVSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFC

Section 30

	(1161)	1161 1170 1180 1190 1200
human coronavirus OC43 S	(1128)	GNGNHIISLVQNAPYGLYFIHFSYVPTKYVTARVSPGLCI
avianinfectiousbronchitisvirusS	(917)	GNGRHVLTIPQNAPNGIVFIHFSYTPDSFVNVTAIVGFCV
bovine coronavirus S	(1138)	GNGNHILSLVQNAPYGLYFIHFSYVPTKYVTAKVSPGLCI
mouse hepatitis virus S	(1094)	GNGNHILSLVQNAPYGLYFIHFSYVPISFTTANVSPGLCI
Consensus	(1161)	GNGNHIISLVQNAPYGLYFIHFSYVPTSFVTAKVSPGLCI

FIGURE 4F (contd.)

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                                                                    Section 31
                                (1201) 1201      ,1210      ,1220      ,1230      1240
    human coronavirus OC43 S (1168) AGDRG------IAPKSGYFVNVNNTWMYTGSGYYYPEPIT
avianinfectiousbronchitisvirusS  (957) KPANASQYAIVPANGRGIFIQVNGSYYITARDMYMPRAIT
        bovine coronavirus S (1178) AGDRG------IAPKSGYFVNVNNTWMFTGSGYYYPEPIT
     mouse hepatitis virus S (1134) SGDRG------LAPKAGYFVQDDGEWKFTGSSYYYPEPIT
                   Consensus (1201) AGDRG      IAPKSGYFVNVNNTWMFTGSGYYYPEPIT
                                                                    Section 32
                                (1241) 1241      ,1250      ,1260      ,1270      1280
    human coronavirus OC43 S (1202) ENNVVVMSTCAVNYTKAPYVMLNTSIP-NLPDFKEELDQW
avianinfectiousbronchitisvirusS  (997) AGDVVTLTSCQANYVSVNKTVITTFVDNDDFDFNDELSKW
        bovine coronavirus S (1212) GNNVVVMSTCAVNYTKAPDVMLNISTP-NLPYFKEELDQW
     mouse hepatitis virus S (1168) DKNSVIMSSCAVNYTKAPEVFLNTSIP-NPPDFKEELDKW
                   Consensus (1241) ENNVVVMSSCAVNYTKAPDVMLNTSIP NLPDFKEELDQW
                                                                    Section 33
                                (1281) 1281      ,1290      ,1300      ,1310      1320
    human coronavirus OC43 S (1241) FKNQTSVAPDLSLDY--INVTFLDLQVEMNRLQEAIKVLN
avianinfectiousbronchitisvirusS (1037) WNDTKHELPDFDKFN--YTVPILDIDSEIDRIQGVIQGLN
        bovine coronavirus S (1251) FKNQTSVAPDLSLDY--INVTFLDLQDEMNRLQEALKVLN
     mouse hepatitis virus S (1207) FKNQTSIAPDLSLDFEKLNVTLLDLTYEMNRIQDAIKKLN
                   Consensus (1281) FKNQTSVAPDLSLDY  INVTFLDLQ EMNRIQEAIKVLN
                                                                    Section 34
                                (1321) 1321      ,1330      ,1340      ,1350      1360
    human coronavirus OC43 S (1279) QSYINLKDIGTYEYYVKWPWYVWLLICLAGVAMLVLLFFI
avianinfectiousbronchitisvirusS (1075) DSLIDLEKLSILKTYIKWPWYVWLAIAFATIIFILILGWV
        bovine coronavirus S (1289) QSYINLKDIGTYEYYVKWPWYVWLLIGFAGVAMLVLLFFI
     mouse hepatitis virus S (1247) ESYINLKEVGTYEMYVKWPWYVWLLIGLAGVAVCVLLFFI
                   Consensus (1321) QSYINLKDIGTYEYYVKWPWYVWLLIGLAGVAMLVLLFFI
                                                                    Section 35
                                (1361) 1361      ,1370      ,1380      ,1390      1400
    human coronavirus OC43 S (1319) CCCTGCG----------TSCFKKCGGCCDDYTGYQELVIKT
avianinfectiousbronchitisvirusS (1115) FFMCGCCGCCCCGCFGIMPLMSKCGKKSSYYTTFDNDVVTE
        bovine coronavirus S (1329) CCCTGCG----------TSCFKKCGGCCDDYTGHQELVIKT
     mouse hepatitis virus S (1287) CCGTCCG----------SCCFKKCGNCCDEYGGHQDSIVIH
                   Consensus (1361) CCCTGCG          TSCFKKCGGCCDDYTGHQELVIKT
                                                                    Section 36
                                (1401) 1401   1408
    human coronavirus OC43 S (1350) SHDD----   SEQ ID NO: 9918
avianinfectiousbronchitisvirusS (1155) QMRPKKSV   SEQ ID NO: 9909
        bovine coronavirus S (1360) SHED----   SEQ ID NO: 9891
     mouse hepatitis virus S (1318) NISSHED-   SEQ ID NO: 9902
                   Consensus (1401) SHDD
```

FIGURE 5

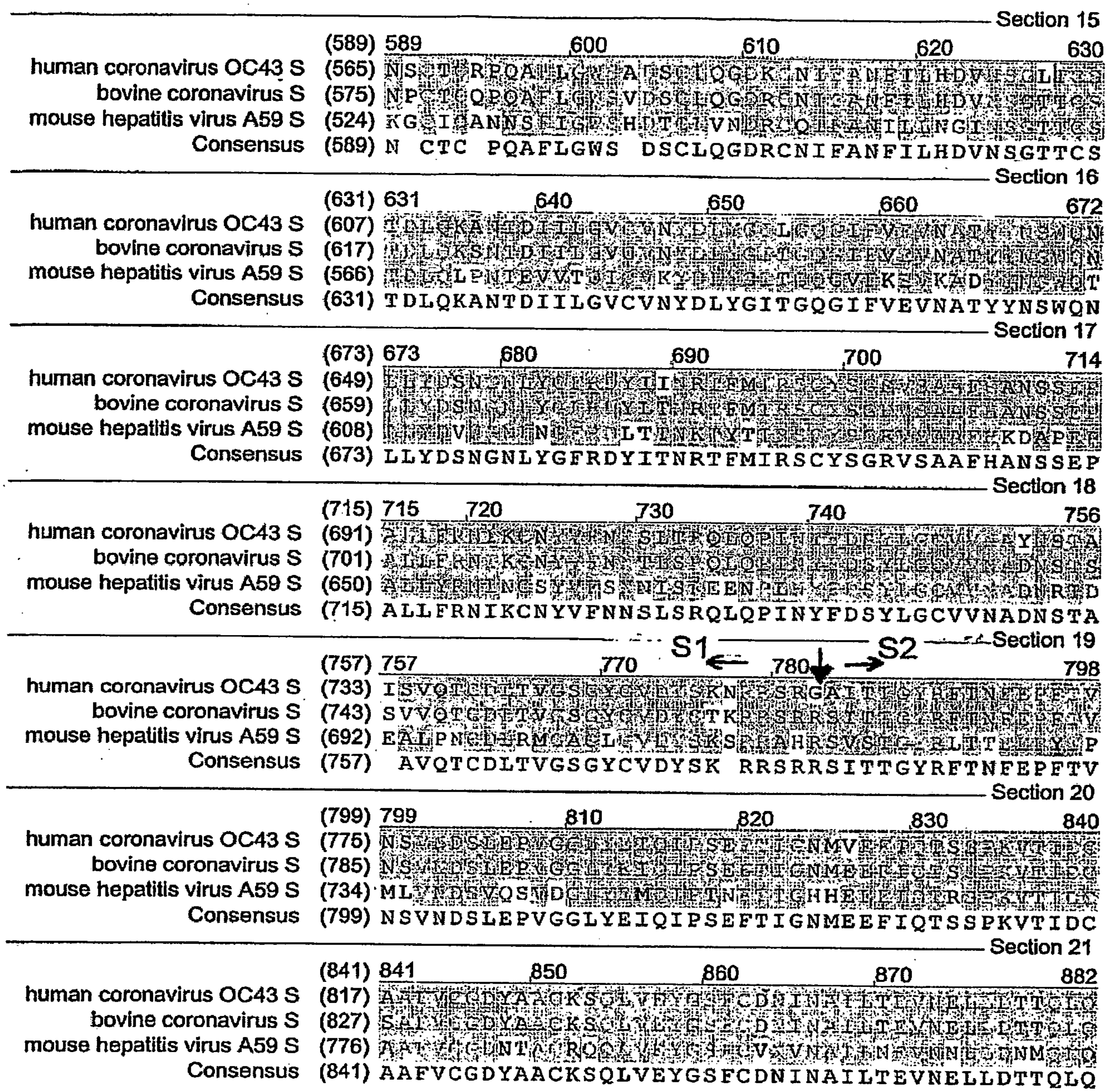
Section 15
(589) 589 600 610 620 630
human coronavirus OC43 S (565)
bovine coronavirus S (575)
mouse hepatitis virus A59 S (524)
Consensus (589) N CTC PQAFLGWS DSCLQGDRCNIFANFILHDVNSGTTCS
Section 16
(631) 631 640 650 660 672
human coronavirus OC43 S (607)
bovine coronavirus S (617)
mouse hepatitis virus A59 S (566)
Consensus (631) TDLQKANTDIILGVCVNYDLYGITGQGIFVEVNATYYNSWQN
Section 17
(673) 673 680 690 700 714
human coronavirus OC43 S (649)
bovine coronavirus S (659)
mouse hepatitis virus A59 S (608)
Consensus (673) LLYDSNGNLYGFRDYITNRTFMIRSCYSGRVSAAFHANSSEP
Section 18
(715) 715 720 730 740 756
human coronavirus OC43 S (691)
bovine coronavirus S (701)
mouse hepatitis virus A59 S (650)
Consensus (715) ALLFRNIKCNYVFNNSLSRQLQPINYFDSYLGCVVNADNSTA
S1 S2 Section 19
(757) 757 770 780 798
human coronavirus OC43 S (733)
bovine coronavirus S (743)
mouse hepatitis virus A59 S (692)
Consensus (757) AVQTCDLTVGSGYCVDYSK RRSRRSITTGYRFTNFEPFTV
Section 20
(799) 799 810 820 830 840
human coronavirus OC43 S (775)
bovine coronavirus S (785)
mouse hepatitis virus A59 S (734)
Consensus (799) NSVNDSLEPVGGLYEIQIPSEFTIGNMEEFIQTSSPKVTIDC
Section 21
(841) 841 850 860 870 882
human coronavirus OC43 S (817)
bovine coronavirus S (827)
mouse hepatitis virus A59 S (776)
Consensus (841) AAFVCGDYAACKSQLVEYGSFCDNINAILTEVNELLDTTQLQ

FIGURE 6

FIGURE 6A

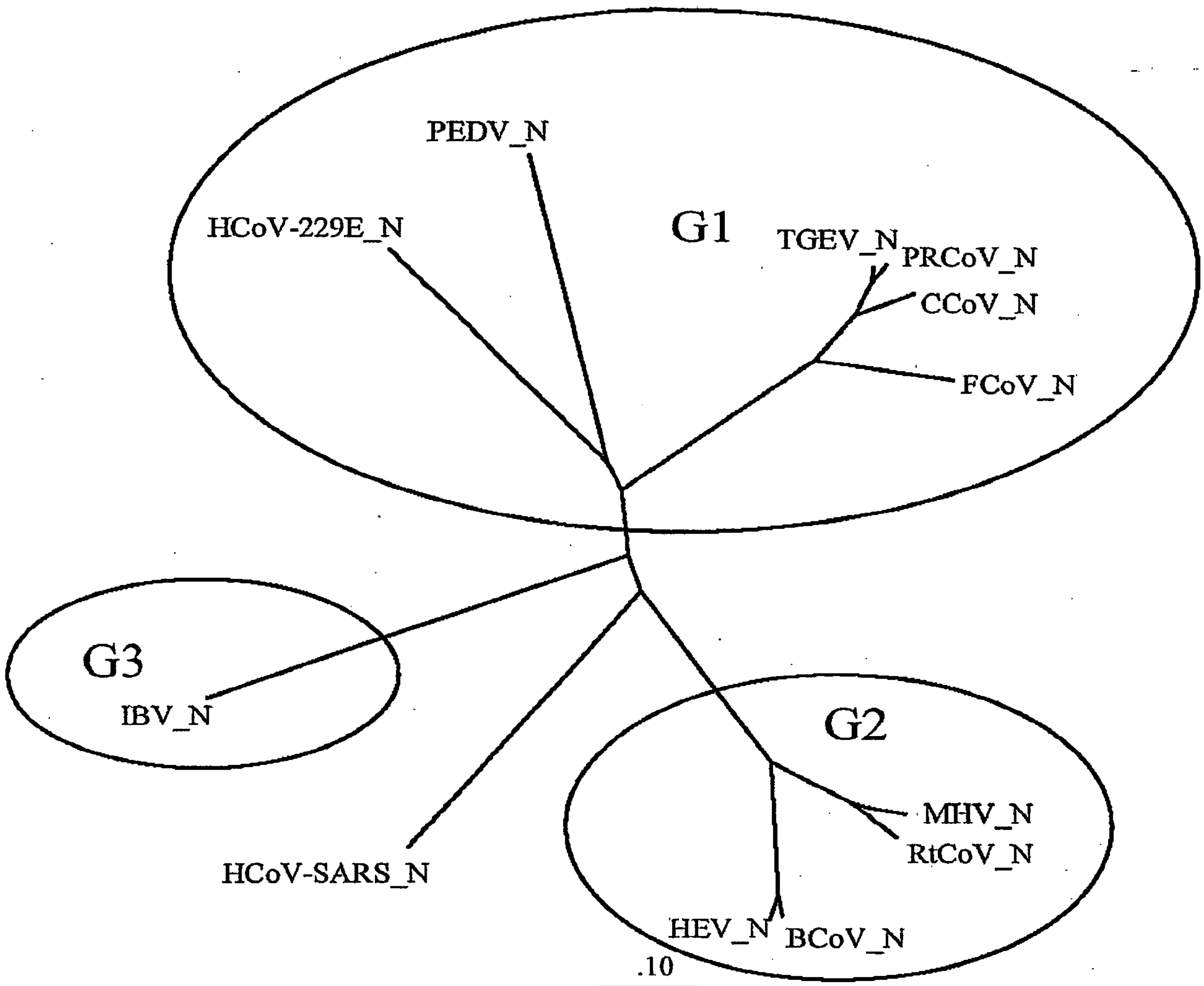

FIGURE 6B

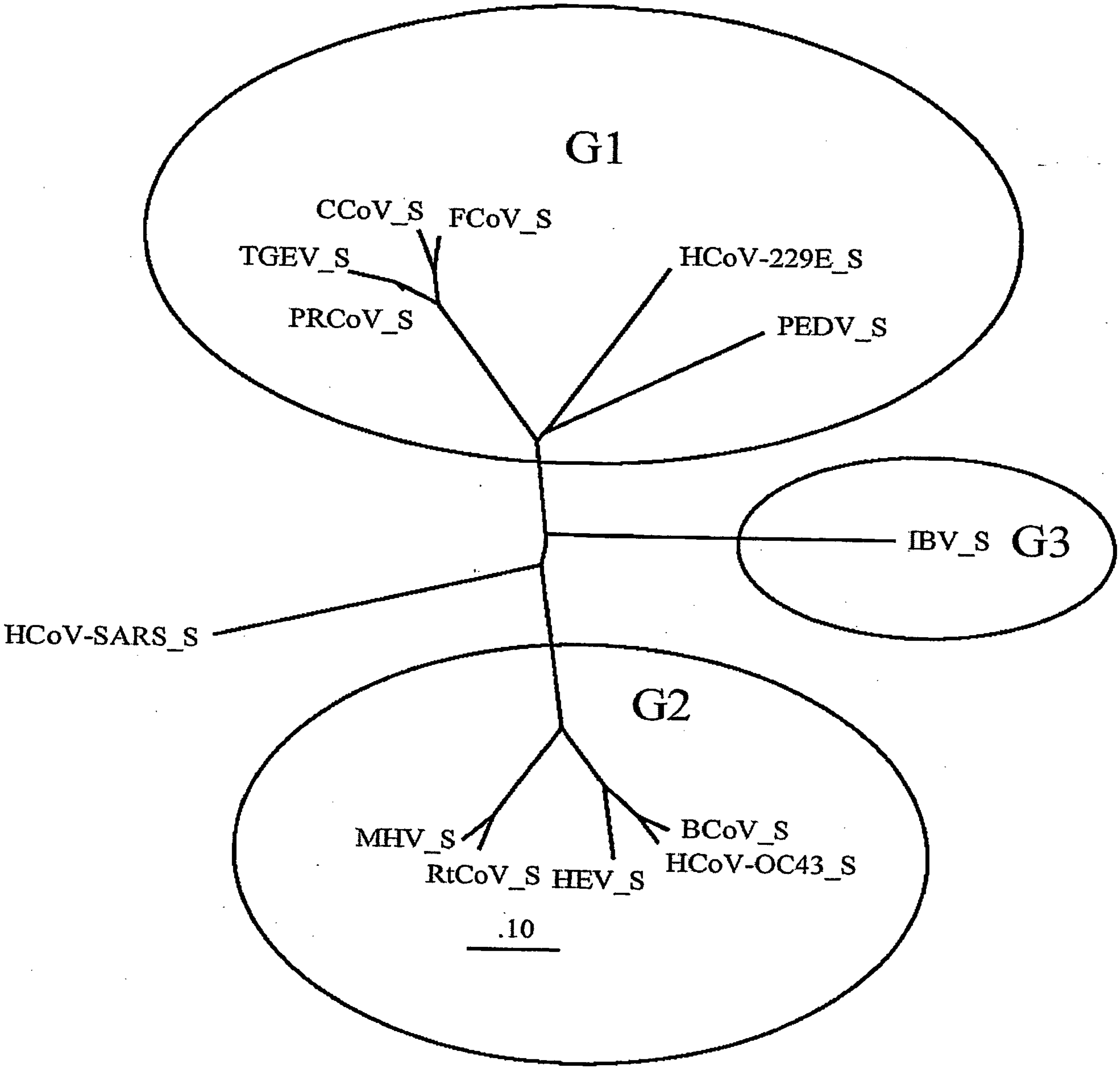
G1
CCoV_S
FCoV_S
TGEV_S
HCoV-229E_S
PRCoV_S
PEDV_S
IBV_S
G3
HCoV-SARS_S
G2
MHV_S
BCoV_S
HCoV-OC43_S
RtCoV_S
HEV_S
.10

FIGURE 6C

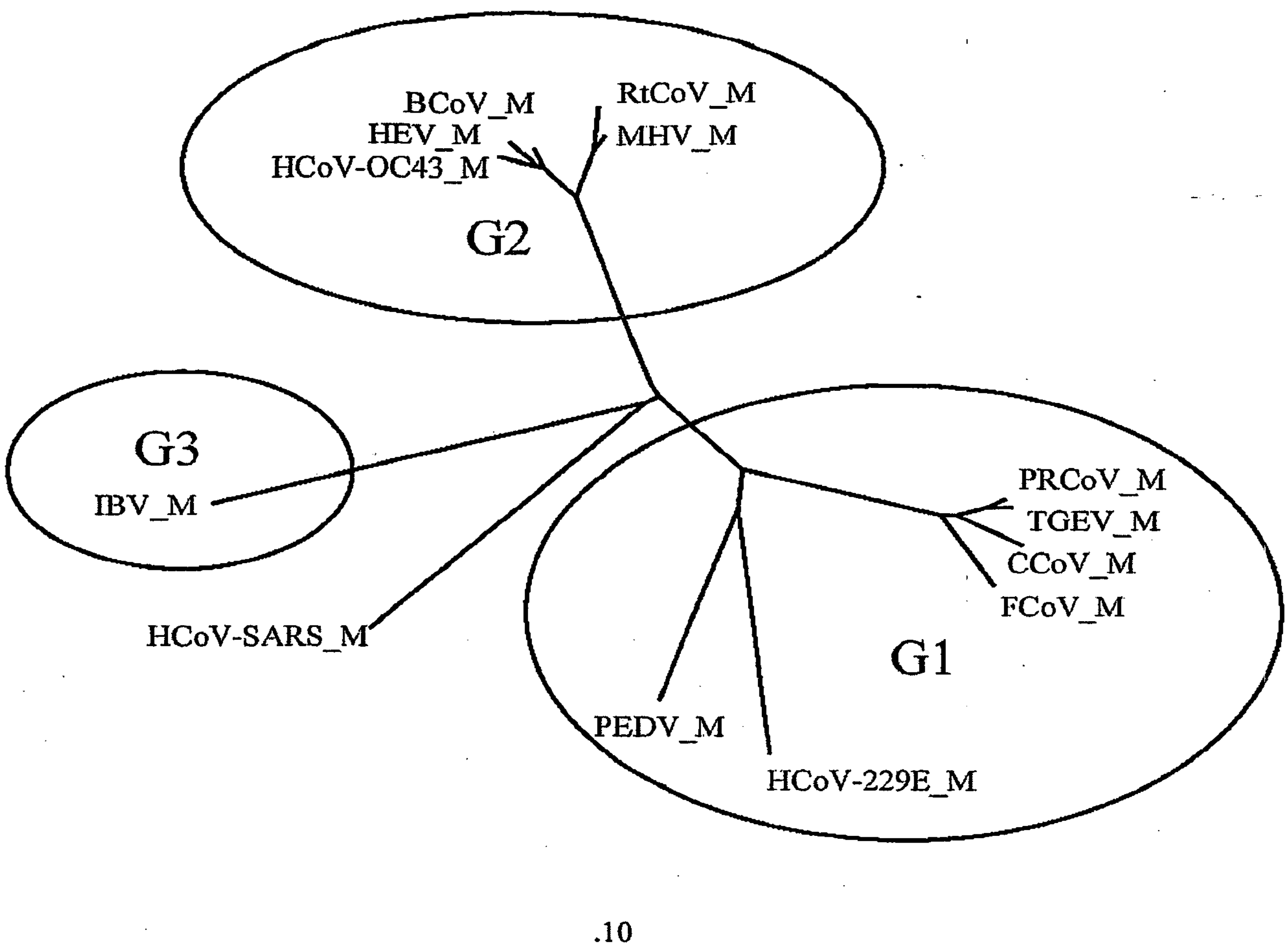
BCoV_M
HEV_M
HCoV-OC43_M
RtCoV_M
MHV_M
G2
G3
IBV_M
HCoV-SARS_M
PRCoV_M
TGEV_M
CCoV_M
FCoV_M
G1
PEDV_M
HCoV-229E_M
.10

FIGURE 7

FIGURE 7A

```
SEQ ID NO:6053  ------MFVLLVAYALLH------------------------------------------ 12
SEQ ID NO:6057  ---MKKLFVVLVVMPLIYGDNFPCSKLTNRTIGNQWNLIETFLLNYSSRLPPNSDVVLGD 60
SEQ ID NO:6061  ---MRSLIYFWLLLPVLPTLSLPQDVTRCQSTTNFRRFFSKFN------VQAPAVVVLGG 52
SEQ ID NO:6065  -----MFLILLISLPTAFAVIGDLKCTTVS-INDVDTGVPSIS----------------- 38
SEQ ID NO:6069  -----MLFVFILFLPSCLGYIGDFRCIQLVNSNGANVSAPSIS----------------- 40
SEQ ID NO:6042  ------MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRG--------------- 39
SEQ ID NO:6072  -----MLVTPLLLVTLLCALCSAVLYDSSS------------------------------ 27
                       :.   :  .

SEQ ID NO:6053  ------------------------------------------------------------
SEQ ID NO:6057  YFPTVQPWFNCIRNDSNDLYVTLENLKALYWDYATENITWNHRQRLNVVVNGYPYSITVT 120
SEQ ID NO:6061  YLPSMNSSSWYCGTGIETASGVHGIFLSYIDSGQGFEIGISQEP-----FDPSGYQLYLH 107
SEQ ID NO:6065  ---------------TDTVDVTNGLGTYYVLDRVYLNTTLLLNG---------------YY 69
SEQ ID NO:6069  ---------------TETVEVSQGLGTYYVLDRVYLNATLLLTG---------------YY 71
SEQ ID NO:6042  ------VYYPDEIFRSDTLYLTQDLFLPFYSNVTGFHTINHTFG---------------NP 79
SEQ ID NO:6072  ------------------------YVYYYQSAFRPPSGWHLQG----------------- 46

SEQ ID NO:6053  ------------IAGCQTTN------------------GLNTSYSVCNG------------ 31
SEQ ID NO:6057  TTRNFNSAEGAIICICKGSPPTTTTESSLTCNWGSECRLNHKFPICPSNSEANCGNMLYG 180
SEQ ID NO:6061  KATNGNTNAIARLRICQFPDNKTLGPTVNDVTTGRNCLFNKAIPAYMRDGKDIVVGITWD 167
SEQ ID NO:6065  PTSGSTYRNMALKGTLLLSTLWFKPPFLSDFTNGIFAKVKNTKVIKDGVMYSEFPAITIG 129
SEQ ID NO:6069  PVDGSKFRNLALTGTNSVSLSWFQPPYLSQFNDGIFAKVQNLKTSTPSGATAYFPTIVIG 131
SEQ ID NO:6042  VIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFA 139
SEQ ID NO:6072  ----------GAYAVVNISSEFNNAGSSSGCTVGIIHGGRVVNASSIAMTAP-------- 88
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SEQ ID NO:6053  ------------------------------------------------------------
SEQ ID NO:6057  LQWFADEVVAYLHGASYRISFENQWSGTVTFGDMRATTLEVAGTLVDLWWFNPVYDVSYY 240
SEQ ID NO:6061  -----NDRVTVFADKIYHFYLKNDWSRVATRCYNRRSCAMQYVYTPTYYMLN-------- 214
SEQ ID NO:6065  ------------------STFVNTSYSVVVQPHTTILGNKLQGFLEISVCQYTMCEYPNT 171
SEQ ID NO:6069  ------------------SLFGYTSYTVVIEPYN---------GVIMASVCQYTICLLPYT 165
SEQ ID NO:6042  VS----------------KPMGTQTHTMIFDNAFN------------------------- 158
SEQ ID NO:6072  ------------------------------------------------------------

SEQ ID NO:6053  -----------------CVGYSENVFAVESGGYIPSDFAFNNWFLLTNTSSVVDGVVRSF 74
SEQ ID NO:6057  RVNNKNGTTVVSNCTDQCASYVANVFTTQPGGFIPSDFSFNNWFLLTNSSTLVSGKLVTK 300
SEQ ID NO:6061  VTSAGEDGIYYEPCTANCTGYAANVFATDSNGHIPEGFSFNNWFLLSNDSTLLHGKVVSN 274
SEQ ID NO:6065  ICNPN-LGNQRVELWHWDTGVVSCLYKRNFTYDVNADYLYFHFYQEGGTFYAYFTDTGVV 230
SEQ ID NO:6069  DCKPNTNGNKLIGFWHTDVKPPICVLKRNFTLNVNADAFYFHFYQHGGTFYAYYADKPSA 225
SEQ ID NO:6042  -CTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLK 217
SEQ ID NO:6072  ---------------SSGMAWSSSQFCTAHCNFSDTTVFVTHCYKHGG--CPLTGMLQQN 131

SEQ ID NO:6053  QPLLLNCLWSVSGLRFTTGFVVYFNGTGRGDCKGFSSDVLSDVIRYNLNFEENLRRG---T 131
SEQ ID NO:6057  QPLLVNCLWPVPSFEEAASTFCFEGAGFDQCNGAVLNNTVDVIRFNLNFTTNVQSGKGAT 360
SEQ ID NO:6061  QPLLVNCLLAIPKIYGLGQFFSFNHTMDGVCNGAAVDRAPEALRFNINDTSVILAEG--S 332
SEQ ID NO:6065  TKFLFNVYLGTVLSHYYVMPLTCN----SALTLEYWVTPLTSKQYLLAFNQDGVIFNAVD 286
SEQ ID NO:6069  TTFLFSVYIGDILTQYYVLPFICNPTAGSTFAPRYWVTPLVKRQYLFNFNQKGVITSAVD 285
SEQ ID NO:6042  PIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVD 277
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SEQ ID NO:6072  LIRVSAMKNGQLFYNLTVSVAKYPTFRSFQCVNNLTSVYLNGDLVYTSNETIDVTSAGVY 191

SEQ ID NO:6053  ILFKTSYG----VVVFYCTNNT-LVSGDAHIPFGTVLGNFYCFVNTTIGNETTSAFVGAL 186
SEQ ID NO:6057  VFSLNTTGGVTLEISCYTVSDS-SFFSYGEIPFGVTDGPRYCYVHY---NGTALKYLGTL 416
SEQ ID NO:6061  IVLHTALG---TNLSFVCSNSSDPHLAIFAIPLGATEVPYYCFLKVDTYNSTVYKFLAVL 389
SEQ ID NO:6065  CKSDFMSEIKCKTLSIAPSTGVYELNGYTVQPIADVYRRIPNLPDCN-IEAWLNDKSVPS 345
SEQ ID NO:6069  CASSYTSEIKCKTQSMLPSTGVYELSGYTVQPVGVVYRRVANLPACN-IEEWLTARSVPS 344
SEQ ID NO:6042  CSQNPLAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVR-FPNITNLCPFGEVFNATKFPS 336
SEQ ID NO:6072  FKAGGPITYKVMREVKALAYFVNGTAQDVILCDGSPRGLLACQYNTGNFSDGFYPFTNSS 251
                                                 .

SEQ ID NO:6053  PKTVREFVISRTGHFYINGYRYFTLGNVEAVNFNVT----TAETTDFCTVALASYADVLV 242
SEQ ID NO:6057  PPSVKEIAISKWGHFYINGYNFFSTFPIDCISFNLT----TGDSDVFWTIAYTSYTEALV 472
SEQ ID NO:6061  PPTVREIVITKYGDVYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVDALI 449
SEQ ID NO:6065  PLNWERKTFSNCNFNMSSLMSFIQAYSFTCNNIDAA----KIYGMCFSSITIDKFAIPNG 401
SEQ ID NO:6069  PLNWERKTFQNCNFNLSSLLRYVQAESLFCNNIDAS----KVYGRCFGSISVDKFAVPRS 400
SEQ ID NO:6042  VYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSAT----KLNDLCFSNVYADSFVVKGD 392
SEQ ID NO:6072  LVKQKFIVYRENSVNTTCTLHNFIFHNETGANPNPSG--VQNIQTYQTKTAQSGYYNFNF 309
                    .     .                      . :            .     :

SEQ ID NO:6053  NVSQTSIANIIYCNSVINRLRCDQLSFDVPDGFYSTSP--IQSVELPVSIVSLPVYHKHT 300
SEQ ID NO:6057  QVENTAITKVTYCNSHVNNIKCSQITANLNNGFYPVSS--SEVGLVNKSVVLLPSFYTHT 530
SEQ ID NO:6061  EVQGTSIQRILYCDDPVSQLKCSQVAFDLDDGFYPISSRNLLSHEQPISFVTLPSFNDHS 509
SEQ ID NO:6065  RKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANVS--VSRFNPSTWNRRFGFTEQS 459
SEQ ID NO:6069  RQVDLQLGNSGFLQTANYKIDTAATSCQLHYTLPKNNVT--INNHNPSSWNRRYGFNDAG 458
SEQ ID NO:6042  DVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDAT------STGNYNYKYRYLRHG 446
SEQ ID NO:6072  SFLSSFVVYKESNFMYGSYHPSCKFRLETINNGLWFNSLS------VSIAYGPLQGGCKQS 363
                        .         .         :       .

SEQ ID NO:6053  FIVLYVDFKPQ-SGGGKCFNCYPAGVNITLANFNET---KGPLCVDTSHFTTKYVAVYAN 356
SEQ ID NO:6057  IVNITIGLGMKRSGYGQPIASTLS--NITLPMQDHN---TDVYCIRSDQFS-VYVHSTCK 584
SEQ ID NO:6061  FVNITVS-----AAFGGLSSANLVASDTTINGFSS-------FCVDTRQFTITLFYNVTN 557
SEQ ID NO:6065  VFKPQPAGVFTDHDVVYAQHCFKASTNFCPCKLDGSLCVGNGPGIDAGYKTSGIGTCPAG 519
SEQ ID NO:6069  VFGKN------QHDVVYAQQCFTVRSSYCPC------------------------------ 483
SEQ ID NO:6042  KLRPFER----------------------------------------------------- 453
SEQ ID NO:6072  VFKGRAT----------------------------------------------------- 370
                 .

SEQ ID NO:6053  VGRWS--------------ASINTGNCPFSFGKVNNFVKFGSVCFSLKDIPGGCAMPIVA 402
SEQ ID NO:6057  SALWDNIFKRNCTDVLDATAVIKTGTCPFSFDKLNNYLTFNKFCLSLSPVGANCKFDVAA 644
SEQ ID NO:6061  SYGYVS--------------KSQDSNCPFTLQSVNDYLSFSKFCVSTSLLAGACTIDLFG 603
SEQ ID NO:6065  TNYLTCHNAAQCDCLCTPDPITSKATGPYKCPQTKYLVGIGEHCSGLAIKSDHCG----G 575
SEQ ID NO:6069  ---------------AQPDIVSPCTT---QTKPKSAFVNVGDHCEGLGVLEDNCGNADPH 525
SEQ ID NO:6042  ---------------------------------DISNVPFSPDGKPCTPPALNCYWPLND 480
SEQ ID NO:6072  -------------------------------CCYAYSYGGPSLCKGVYSGELDHNFECGL 399

SEQ ID NO:6053  NWAYSKY---YTIG-------------SLYVSWSDGDGITGVPQPVEGVSSFMNVTLDKC 446
SEQ ID NO:6057  -RTRTNE---QVVR-------------SLYVIYEEGDNIVGVPSDNSGVHDLSVLHLDSC 687
SEQ ID NO:6061  YPAFGSG---VKLT-------------SLYFQFTKGELITGTPKPLEGITDVSFMTLDVC 647
SEQ ID NO:6065  NPCTCQP---QAFLGWSVDSCLQGDRCNIFANFILHDVNSGTTCSTDLQKSNTDIILGVC 632
SEQ ID NO:6069  KGCICAN---NSFIGWSHDTCLVNDRCQIFANILLNGINSGTTCSTDLQLPNTEVVTGIC 582
SEQ ID NO:6042  YGFYTTTGIGY----------------QPYRVVVLSFELLNAPATVCGPKLSTDLIKNQC 524
SEQ ID NO:6072  LVYVTKS--------------------------GGSRIQTATEPPVITQNNYNNITLNTC 433
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SEQ ID NO:6053   TKYNIYDVSGVGVIRVSNDTFLN---------GITYTSTSGNLLGFKDVTKGTIYSITPC 497
SEQ ID NO:6057   TDYNIYGRTGVGIIRQTNRTLLS---------GLYYTSLSGDLLGFKNVSDGVIYSVTPC 738
SEQ ID NO:6061   TKYTIYGFKGEGIITLTNSSILA---------GVYYTSDSGQLLAFKNVTSGAVYSVTPC 698
SEQ ID NO:6065   VNYDLYGITGQGIFVEVNATYYNS-------WQNLLYDSNGNLYGFRDYLTNRTFMIRSC 685
SEQ ID NO:6069   VKYDLYGITGQGVFKEVKADYYNS-------WQTLLYDVNGNLNGFRDLTTNKTYTIRSC 635
SEQ ID NO:6042   VNFNFNGLTGTGVLTPSSKRFQP--------FQQFGRDVSDFTDSVRDPKTSEILDISPC 576
SEQ ID NO:6072   VDYNIYGRTGQGFITNVTDSAVSYNYLADAGLAILDTSGSIDIFVVQGEYGLNYYKVNPC 493
                 ..: :  .  .* *.:     .                  . .     .:.         : .*

SEQ ID NO:6053   NPPDQLVVYQQA--VVGAMLSENFTSYGFSNVVELPKFFYASNGTYN------------- 542
SEQ ID NO:6057   DVSAQAAVIDGT--IVGAITSINSELLGLTHWTTTPNFYYYSIYNYTNDRTRGTAIDSND 796
SEQ ID NO:6061   SFSEQAAYVNDD--IVGVISSLSNS--TFNNTRELPGFFYHSNDGSN------------- 741
SEQ ID NO:6065   YSGRVSAAFHAN--SSEPALLFRNIKCNYVFNNTLSRQLQPINYFDSYLGCVVNADNSTS 743
SEQ ID NO:6069   YSGRVSAAFHKD--APEPALLYRNINCSYVFSNNISREENPLNYFDSYLGCVVNADNRTD 693
SEQ ID NO:6042   AFGGVSVITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCL 636
SEQ ID NO:6072   EDVNQQFVVSGGK-LVGILTSRNETGSQLLENQFYIKITNGTRRFRRSITEN-------- 544

SEQ ID NO:6053   -CTDAVLTYSSFGVCADGS-------IIAVQPRNVSYDSVSAIVTAN----------LSI 584
SEQ ID NO:6057   VDCEPVITYSNIGVCKNGA-------FVFINVTHSDGD-VQPISTGN----------VTI 838
SEQ ID NO:6061   -CTEPVLVYSNIGVCKSGS-------IGYVPSQYGQVK-IAPTVTGN----------ISI 782
SEQ ID NO:6065   SVVQTCDLTVGSGYCVDYSTKRRSRRSITTGYRFTNFEPFTVNSVNDSLEPVGGLYEIQI 803
SEQ ID NO:6069   EALPNCDLRMGAGLCVDYSKSRRADRSVSTGYRLTTFEPYTPMLVNDSVQSVDGLYEMQI 753
SEQ ID NO:6042   IGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAI 696
SEQ ID NO:6072   VANCPYVSYGKFCIKPDGS------IATIVPKQLEQFVAPLFNVTEN----------VLI 588
                                   . .                                   : *

SEQ ID NO:6053   PSNWTTSVQVEYLQITSTPIVVDCSTYVCNGNVRCVELLKQYTSACKTIEDALRNSARLE 644
SEQ ID NO:6057   PTNFTISVQVEYIQVYTTPVSIDCSRYVCNGNPRCNKLLTQYVSACQTIEQALAMGARLE 898
SEQ ID NO:6061   PTNFSMSIRTEYLQLYNTPVSVDCATYVCNGNSRCKQLLTQYTAACKTIESALQLSARLE 842
SEQ ID NO:6065   PSEFTIGNMEEFIQTSSPKVTIDCSAFVCGDYAACKSQLVEYGSFCDNINAILTEVNELL 863
SEQ ID NO:6069   PTNFTIGHHEEFIQTRSPKVTIDCAAFVCGDNTACRQQLVEYGSFCVNVNAILNEVNNLL 813
SEQ ID NO:6042   PTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQ 756
SEQ ID NO:6072   PNSFNLTVTDEYIQTRMDKVQINCLQYVCGSSLDCRKLFQQYGPVCDNILSVVNSVGQKE 648
                 *..:.     *  :          ::*  ::*..   *  .  : :*  .  *    :   :

SEQ ID NO:6053   SADVSEMLTFDKKAFTLANVSSFGDYN----------------LSSVIPSLPTSGS---- 684
SEQ ID NO:6057   NMEVDSMLFVSENALKLASVEAFNSSETLDPIYKEWPNIGGSWLEGLKYILPSHNS---- 954
SEQ ID NO:6061   SVEVNSMLTISEEALQLATISSFNGDG-----------YNFTNVLGASVYDPASGR---- 887
SEQ ID NO:6065   DTTQLQVANSLMNGVTLSTKLKDGVNFN----------VDDINFSPVLGCLGSDCN---- 909
SEQ ID NO:6069   DNMQLQVASALMQGVTISSRLPDGISGP----------IDDINFSPLLGCIGSTCAEDGN 863
SEQ ID NO:6042   DRNTREVFAQVKQMYKTPTLKYFGGFN----------------FSQILPDPLKPTK---- 796
SEQ ID NO:6072   DMELLNFYSSTKPAGFNTPVLSNVSTG-------------EFNISLLLTNPSSRRK---- 691
                 .      ..        .                           .

SEQ ID NO:6053   ---RVAGRSAIEDILFSKLVTSGLGTVDADYKKCTKGLS--IADLACAQYYNGIMVLPGV 739
SEQ ID NO:6057   ---KRKYRSAIEDLLFDKVVTSGLGTVDEDYKRCTGGYD--IADLVCAQYYNGIMVLPGV 1009
SEQ ID NO:6061   ---VVQKRSVIEDLLFNKVVTNGLGTVDEDYKRCSNGRS--VADLVCAQYYSGVMVLPGV 942
SEQ ID NO:6065   ---KVSSRSAIEDLLFSKVKLSDVG-FVEAYNNCTGGAE--IRDLICVQSYNGIKVLPPL 963
SEQ ID NO:6069   GPSAIRGRSAIEDLLFDKVKLSDVG-FVEAYNNCTGGQE--VRDLLCVQSFNGIKVLPPV 920
SEQ ID NO:6042   -------RSFIEDLLFNKVTLADAG-FMKQYGECLGDIN--ARDLICAQKFNGLTVLPPL 846
SEQ ID NO:6072   -------RSLIEDLLFTSVESVGLP-TNDAYKNCTAGPLGFFKDLACAREYNGLLVLPPI 743
                        ** ***:**  .:    .        *  .*   .       ** *.: :.*: *** :
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SEQ ID NO:6053  ADAERMAMYTGSLIGGIALGGLTS----AVSIPFSLAIQARLNYVALQTDVLQENQKILA 795
SEQ ID NO:6057  ANADKMTMYTASLAGGITLGALGGG---AVAIPFAVAVQARLNYVALQTDVLNKNQQILA 1066
SEQ ID NO:6061  VDAEKLHMYSASLIGGMALGGITA----AAALPFSYAVQARLNYLALQTDVLQRNQQLLA 998
SEQ ID NO:6065  LSENQISGYTLAATSASLFPPWSA----AAGVPFYLNVQYRINGIGVTMDVLSQNQKLIA 1019
SEQ ID NO:6069  LSESQISGYTTGATAAAMFPPWSA----AAGVPFSLSVQYRINGLGVTMNVLSENQKMIA 976
SEQ ID NO:6042  LTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIA 906
SEQ ID NO:6072  ITAEMQALYTSSLVASMAFGGITA----AGAIPFATQLQARINHLGITQSLLLKNQEKIA 799
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SEQ ID NO:6053  ASFNKAMTNIVDAFTGVNDAITQTSQALQTVATALNKIQDVVNQQGNSLNHLTSQLRQNF 855
SEQ ID NO:6057  SAFNQAIGNITQSFGKVNDAIHQTSRGLATVAKALAKVQDVVNIQGQALSHLTVQLQNNF 1126
SEQ ID NO:6061  ESFNSAIGNITSAFESVKEAISQTSKGLNTVAHALTKVQEVVNSQGSALNQLTVQLQHNF 1058
SEQ ID NO:6065  NAFNNALGAIQEGFDATN---------------SALVKIQAVVNANAEALNNLLQQLSNRF 1065
SEQ ID NO:6069  SAFNNALGAIQDGFDATN---------------SALGKIQSVVNANAEALNNLLNQLSNRF 1022
SEQ ID NO:6042  NQFNKAISQIQESLTTTS---------------TALGKLQDVVNQNAQALNTLVKQLSSNF 952
SEQ ID NO:6072  ASFNKAIGHMQEGFRSTS---------------LALQQIQDVVSKQSAILTETMASLNKNF 845
                 **.*:  : ..:  ..                 ** ::* **. :.   *.    .*  .*

SEQ ID NO:6053  QAISSSIQAIYDRLDTIQADQQVDRLITGRLAALNVFVSHTLTKYTEVRASRQLAQQKVN 915
SEQ ID NO:6057  QAISSSISDIYNRLDELSADAQVDRLITGRLTALNAFVSQTLTRQAEVRASRQLAKDKVN 1186
SEQ ID NO:6061  QAISSSIDDIYSRLDILSADVQVDRLITGRLSALNAFVAQTLTKYTEVQASRKLAQQKVN 1118
SEQ ID NO:6065  GAISSSLQEILSRLDALEAQAQIDRLINGRLTALNAYVSQQLSDSTLVKFSAAQAMEKVN 1125
SEQ ID NO:6069  GAISASLQEILTRLEAVEAKAQIDRLINGRLTALNAYISKQLSDSTLIKVSAAQAIEKVN 1082
SEQ ID NO:6042  GAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMS 1012
SEQ ID NO:6072  GAISSVIQEIYQQFDAIQANAQVDRLITGRLSSLSVLASAKQAEYIRVSQQRELATQKIN 905
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SEQ ID NO:6053  ECVKSQSKRYGFCG-NGTHIFSIVNAAPEGLVFLHTVLLPTQYKDVEAWSGLCVDG---- 970
SEQ ID NO:6057  ECVRSQSQRFGFCG-NGTHLFSLANAAPNGMIFFHTVLLPTAYETVTAWPGICASDG-DR 1244
SEQ ID NO:6061  ECVKSQSQRYGFCGGDGEHIFSLVQAAPQGLLFLHTVLVPGDFVNVLAIAGLCVNG---- 1174
SEQ ID NO:6065  ECVKSQSSRINFCG-NGNHIISLVQNAPYGLYFIHFSYVPTKYVTAKVSPGLCIAG---- 1180
SEQ ID NO:6069  ECVKSQTTRINFCG-NGNHILSLVQNAPYGLYFIHFSYVPISFTTANVSPGLCISG---- 1137
SEQ ID NO:6042  ECVLGQSKRVDFCG-KGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEG---- 1067
SEQ ID NO:6072  ECVKSQSIRYSFCG-NGRHVLTIPQNAPNGIVFIHFSYTPDSFVNVTAIVGFCVKPANAS 964
                ***  .*: *  .***  .* *:::: : ** *: *:*     *       .   .:*

SEQ ID NO:6053  TNGYVLRQPNLALYK------EGNYYRITSRIMFEPRIPTMADFVQIENCNVTFVNISRS 1024
SEQ ID NO:6057  TFGLVVKDVQLTLFRN-----LDDKFYLTPRTMYQPRVATSSDFVQIEGCDVLFVNATVS 1299
SEQ ID NO:6061  EIALTLREPGLVLFTHELQTYTATEYFVSSRRMFEPRKPTVSDFVQIESCVVTYVNLTSD 1234
SEQ ID NO:6065  DRGIAPKSGYFVNVNN--------TWMFTGSGYYYPEPITGNNVVVMSTCAVNYTKAPDV 1232
SEQ ID NO:6069  DRGLAPKAGYFVQDDG--------EWKFTGSSYYYPEPITDKNSVIMSSCAVNYTKAPEV 1189
SEQ ID NO:6042  -KAYFPREGVFVFNGT--------SWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNT 1118
SEQ ID NO:6072  QYAIVPANGRGIFIQVN------GSYYITARDMYMPRAITAGDVVTLTSCQANYVSVNKT 1018
                   .                          : .:     : *.   *   :  .     * .

SEQ ID NO:6053  ELQTIVP-EYIDVNKTLQELSYKL-PNYTVPDLVVEQYNQTILNLTSEISTLENKSAELN 1082
SEQ ID NO:6057  DLPSIIP-DYIDINQTVQDILENFRPNWTVPELTFDIFNATYLNLTGEIDDLEFRSEKLH 1358
SEQ ID NO:6061  QLPDVIP-DYIDVNKTLDEILASL-PNRTGPSLPLDVFNATYLNLTGEIADLEQRSESLR 1292
SEQ ID NO:6065  MLNISTP-NLPYFKEELDQWFKNQTSVAPDLSLDY--INVTFLDLQDEMN---------- 1279
SEQ ID NO:6069  FLNTSIP-NPPDFKEELDKWFKNQTSIAPDLSLDFEKLNVTLLDLTYEMN---------- 1238
SEQ ID NO:6042  VYDPLQP-ELDSFKEELDKYFKNHTSPDVDLGDISG-INASVVNIQKEID---------- 1166
SEQ ID NO:6072  VITTFVDNDDFDFNDELSKWWNDTKHELPDFDKFN--YTVPILDIDSEID---------- 1066
                     :   .:. :..   .               . . :::  *:
```

```
SEQ ID NO:6053  YTVQKLQTLIDNINSTLVDLKWLNRVETYIKWPWWVWLCISVVLIFVVSMLLLCCCSTGC 1142
SEQ ID NO:6057  NTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVVIFCIPLLLFCCCSTGC 1418
SEQ ID NO:6061  NTTEELRSLINNINNTLVDLEWLNRVETYIKWPWWVWLIIVIVLIFVVSLLVFCCISTGC 1352
SEQ ID NO:6065  ----RLQEAIKVLNQSYINLKDIGTYEYYVKWPWYVWLLIGFAGVAMLVLLFFICCCTGC 1335
SEQ ID NO:6069  ----RIQDAIKKLNESYINLKEVGTYEMYVKWPWYVWLLIGLAGVAVCVLLFFICCCTGC 1294
SEQ ID NO:6042  ----RLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSC 1222
SEQ ID NO:6072  ----RIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWLAIAFATIIFILILGWVFFMTGC 1122
                      .:     . :*.: ::*: :.   : *:****:*** :      :    :      *.*

SEQ ID NO:6053  CGFFSCFA-------SSIRGCCESTKLPYYD-VEKIHIQ--- 1173
SEQ ID NO:6057  CGCIGCLG-------SCCHSICSRRQFENYEPIEKVHVH--- 1450
SEQ ID NO:6061  CGCCGCCG-------ACFSGCCRGPRLQPYEAFEKVHVQ--- 1384
SEQ ID NO:6065  G--TSCFK-------KCGGCCDDYTGHQELVIKT---SHED- 1364
SEQ ID NO:6069  G--SCCFK-------KCGNCCDEYGGHQDSIVIHNISSHED- 1326
SEQ ID NO:6042  CSCLKGAC-------SCG-SCCKFDEDDSEPVLKGVKLHYT- 1255
SEQ ID NO:6072  CGCCCGCFGIMPLMSKCGKKSSYYTTFDNDVVTEQYRPKKSV 1164
```

FIGURE 7B

```
SEQ ID NO:6054  --------MFLKLVDDHA-LVVNVLLWCVVLIVILLVCITIIKLIKLCFTCHMFCNRTVY 51
SEQ ID NO:6062  ---------MLQLVNDNG-LVVNVILWLFVLFFLLIISITFVQLVNLCFTCHRLCNSAVY 54
SEQ ID NO:6058  ----MTFPRALTVIDDNG-MVINIIFWFLLIIILILLSIALLNIIKLCMVCCNLGRTVII 59
SEQ ID NO:6045  --------MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLV 52
SEQ ID NO:6073  ------MNLLNKSLEENG-SFLTALYIIVGFLALYLLGRALQAFVQAADACCLFWYTWVV 57
SEQ ID NO:6066  ------MFMADAYFADTVWYVGQIIFIVAICLLVIIVVVAFLATFKLCIQLCGMCNTLVL 54
                                   :     .    :         . . ::  ::   . . .     :    :

SEQ ID NO:6054  GP----IKNVYH-IY-QSYMH-------IDPF---------PKRVIDF------- 77
SEQ ID NO:6062  TP----IGRLYR-VY-KSYMR-------IDPL---------PSTVIDV------- 80
SEQ ID NO:6058  VP----AQHAYD-AY-KNFMR-------IKAYN--------PDGALLA------- 86
SEQ ID NO:6045  KP----TVYVYS-RV-KNLNS-------SEGV---------PDLLV--------- 76
SEQ ID NO:6073  IPGAKGTAFVYKYTYGRKLNNPELEAVIVNEFPKNGWNNKNPANFQDAQRDKLYS 112
SEQ ID NO:6066  SP----SIYVFN-RG-RQFYEF------YNDVKP-------PVLDVDDV------ 84
                 *           :       :.          .             *
```

FIGURE 7C

```
SEQ ID NO:6055  -------------------------MSNDNC----------------TGDIVTHLKNWNF 19
SEQ ID NO:6063  -------------------------MSNGSIP---------------VDEVIEHLRNWNF 24
SEQ ID NO:6059  ----MKILLILACVIACACGERYCAMKSDTDLSCRNSTASDCESCFNGGDLIWHLANWNF 60
SEQ ID NO:6067  -------------------------MSSVT-TPAPVYTWT-------ADEAIKFLKEWNF 27
SEQ ID NO:6070  -------------------------MSSTTQAPEPVYQWT-------ADEAVQFLKEWNF 33
SEQ ID NO:6046  -------------------------MADNGTIT--------------VEELKQLLEQWNL 21
SEQ ID NO:6074  -------------------------MPNETNCTLD------------FEQSVQLFKEYNL 28
                                          * .                           :    : ::*:

SEQ ID NO:6055  GWNVILTIFIVILQFGHYKYSRLFYGLKMLVLWLLWPLVLALSIFDTWANWDSN-WAFVA 78
SEQ ID NO:6063  TWNIILTILLVVLQYGHYKYSVFLYGVKMAILWILWPLVLALSLFDAWASFQVN-WVFFA 83
SEQ ID NO:6059  SWSIILIVFITVLQYGRPQFSWFVYGIKMLIMWLLWPVVLALTIFNAYSEYQVSRYVMFG 120
SEQ ID NO:6067  SLGIILLFITVILQFGYTSRSMFVYVIKMVILWLMWPLTIILTIFN--CVYALN-NVYLG 84
SEQ ID NO:6070  SLGIILLFITIILQFGYTSRSMFIYVVKMIILWLMWPLTIVLCIFN--CVYALN-NVYLG 90
SEQ ID NO:6046  VIGFLFLAWIMLLQFAYSNRNRFLYIIKLVFLWLLWPVTLACFVLA--AVYRIN-WVTGG 78
```

```
SEQ ID NO:6074  FITAFLLFLTIILQYGYATRSKVIYTLKMIVLWCFWPLNIAVGVIS--CTYPPN-TGGLV 85
                   ::      :**:.    . ..* :*: .:* :**: :    ::    . :  .

SEQ ID NO:6055  FSFFMAVSTLVMWVMYFANSFRLFRRARTFWAWNPEVNAITVTTVL-GQTYYQPIQQAPT 137
SEQ ID NO:6063  FSILMACITLMLWIMYFVNSIRLWRRTHSWWSFNPETDALLTTSVM-GRQVCIPVLGAPT 142
SEQ ID NO:6059  FSIAGAIVTFVLWIMYFVRSIQLYRRTKSWWSFNPETKAILCVSAL-GRSYVLPLEGVPT 179
SEQ ID NO:6067  FSIVFTIVAIIMWIVYFVNSIRLFIRTGSWWSFNPETNNLMCIDMK-GRMYVRPIIEDYH 143
SEQ ID NO:6070  FSIVFTIVSIVIWIMYFVNSIRLFIRTGSWWSFNPETNNLMCIDMK-GTVYVRPIIEDYH 149
SEQ ID NO:6046  IAIAMACIVGLMWLSYFVASFRLFARTRSMWSFNPETNILLNVPLR-GTIVTRPLMESEL 137
SEQ ID NO:6074  AAIILTVFACLSFVGYWIQSIRLFKRCRSWWSFNPESNAVGSILLTNGQQCNFAIESVPM 145
                 ::  :    : :: *:  *::*: *  : *::*** . :         *     .:

SEQ ID NO:6055  GITVTLLSGVLYVDGHRLASGVQVHNLPEYMTVAVPSTTIIYSRVGRSVNSQNSTG--WV 195
SEQ ID NO:6063  GVTLTLLSGTLLVEGYKVATGVQVSQLPNFVTVAKATTTIVYGRVGRSVNASSGTG--WA 200
SEQ ID NO:6059  GVTLTLLSGNLYAEGFKIAGGMNIDNLPKYVMVALPSRTIVYTLVGKKLKASSATG--WA 237
SEQ ID NO:6067  TLTVTIIRGHLYMQGIKLGTGYSLSDLPAYVTVAKVSHLLTYKRG-FLDKIGDTSG--FA 200
SEQ ID NO:6070  TLTATIIRGHLYMQGVKLGTGFSLSDLPAYVTVAKVSHLCTYKRA-FLDKVDGVSG--FA 206
SEQ ID NO:6046  VIGAVIIRGHLRMAGHSLGR-CDIKDLPKEITVATS-RTLSYYKLGASQRVGTDSG--FA 193
SEQ ID NO:6074  VLSPIIKNGVLYCEGQWLAK-CEPDHLPKDIFVCTPDRRNIYRMVQKYTGDQSGNKKRFA 204
                 :  :  * *   *  :.   .  .**  : *.       *           .   :.

SEQ ID NO:6055  FYVRVKHGDFSAVSSPMSNMTENERLLHFF 225
SEQ ID NO:6063  FYVRSKHGDYSAVSNPSAVLTDSEKVLHLV 230
SEQ ID NO:6059  YYVKSKAGDYSTEAR-TDNLSEQEKLLHMV 266
SEQ ID NO:6067  VYVKSKVGNYRLPSTQKGSGLDTALLRNNI 230
SEQ ID NO:6070  VYVKSKVGNYRLPSN-KPSGADTALLR--I 233
SEQ ID NO:6046  AYNRYRIGNYKLNTDHAGSNDNIALLVQ-- 221
SEQ ID NO:6074  TFVYAKQSVDTGELESVATGGSSLYT---- 230
                 :  : .          .
```

FIGURE 7D

```
SEQ ID NO:6056  ------------------------MATVKWADASE-----PQRGRQG------------- 18
SEQ ID NO:6064  -----------------------------MASVSF-----QDRGRK-------------- 17
SEQ ID NO:6060  --------------------MANQGQRVSWGDEST-----KTRGRSNSRGRKN------- 31
SEQ ID NO:6068  MSFTPGKQSSS-RASSGNRSGNGILK---WADQSDQSRNVQTRGRR-AQPKQTATSQQPS 55
SEQ ID NO:6071  MSFVPGQENAGGRSSSVNRAGNGILKKTTWADQTERGPNNQNRGRR-NQPKQTATTQ-PN 58
SEQ ID NO:6051  ------------MSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPN 48
SEQ ID NO:6075  -----------MASGKAAGKTDAPAPVIKLGGPKP-----PKVGSS-------------- 35
                                         . . .      *

SEQ ID NO:6056  ----RIPYSLYSPLLVDSEQPW-KVIPRNLVPINKK-DKNKLIGYWN--VQKRFRTRKGK 70
SEQ ID NO:6064  ----RVPLSLYAPLRVTNDKPLSKVLANNAVPTNKG-NKDQQIGYWN--EQIRWRMRRGE 70
SEQ ID NO:6060  ---NNIPLSFFNPITLQQGSKFWNLCPRDFVPKGIG-NRDQQIGYWN--RQTRYRMVKGQ 85
SEQ ID NO:6068  GGNVVPYYSWFSGITQFQKGKEFEFAEGQGVPIAPGVPATEAKGYWYRHNRRSFKTADGN 115
SEQ ID NO:6071  SGSVVPHYSWFSGITQFQKGKEFQFAEGQGVPIANGIPASEQKGYWYRHNRRSFKTPDGQ 118
SEQ ID NO:6051  NT-----ASWFTALTQHGK-EELRFPRGQGVPINTNSGPDDQIGYYRRATRR-VRGGDGK 101
SEQ ID NO:6075  -----GNASWFQAIKAKKLNTPPPKFEGSGVPDNENIKPSQQHGYWR--RQARFKPGKGG 88
                      * :  :              . **        . **:    :   :    *

SEQ ID NO:6056  RVDLSPKLHFYYLGTGPHKDAKFRERVEGVVWVAVDGAKTEPTGYGVRRKNSEPEIPHFN 130
SEQ ID NO:6064  RIEQPSNWHFYYLGTGPHGDLRYRTRTEGVFWVAKEGAKTEPTNLGVRKASEKPIIPKFS 130
SEQ ID NO:6060  RKELPERWFFYYLGTGPHADAKFKDKLDGVVWVAKDGAMNKPTTLGSRGANNESKALKFD 145
SEQ ID NO:6068  QRQLLPRWYFYYLGTGPHAKDQYGTDIDGVYWVASNQADVNTPADILDRDPSSD--EAIP 173
```

```
SEQ ID NO:6071  QKQLLPRWYFYYLGTGPHAGASYGDSIEGVFWVANSQADTNTRSDIVERDPSSH--EAIP 176
SEQ ID NO:6051  MKELSPRWYFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNN--AATV 159
SEQ ID NO:6075  RKPVPDAWYFYYTGTGPAADLNWGDTQDGIVWVAAKGADTKSRSNQGTRDPDKF--DQYP 146
                      .*** ****       :    :*: *** . *   .          ..

SEQ ID NO:6056  QKLPNGVTVVEE---PDSRAPSRSQSR-----------------SQSRGRGESK------- 164
SEQ ID NO:6064  QQLPSVVEIVEPNTPPASRANSRSRSRGNGNNRSRSPSNNRGNNQSRGNSQNRGNNQGRG 190
SEQ ID NO:6060  GKVPGEFQLEVN----QSRDNSRSRSQ------------------SRSRSRNR------- 176
SEQ ID NO:6068  TRFPPGTVLPQGYYIEGS-GRSAPNSR-----------------STSRASSRASS------ 210
SEQ ID NO:6071  TRFAPGTVLPQGFYVEGS-GRSAPASR-----------------SGSRSQSRGP------- 212
SEQ ID NO:6051  LQLPQGTTLPKGFYAEGSRGGSQASSR-----------------SSSRSRGNSR------- 196
SEQ ID NO:6075  LRFSDGGPDGNFRWDFIPLNRGRSGRS-----------------TAASSAAASR------- 183
                 :..         .   . .                        : . .

SEQ ID NO:6056  -------------PQSRNPSSDRNHN-------SQDDIMKAVAAALKSLGFDKPQEKDKKS 205
SEQ ID NO:6064  ASQNRGGNNNNNNKSRNQSNNRNQSNDRGGVTSRDDLVAAVKDALKSLGIGENPDRHKQ- 249
SEQ ID NO:6060  -------------SQSRGRQQFNNKK--------DDSVEQAVLAALKKLGVDTEKQQRS- 215
SEQ ID NO:6068  -------------AGSRSRANSGNRT-------PTSGVTPDMADQIASLVLAKLGKDAAKP 251
SEQ ID NO:6071  --------------NNRARSSSNQRQ-------PASTVKPDMAEEIAALVLAKLGKDAGQP 252
SEQ ID NO:6051  --------------NSTPGSSRGNS-------PARMASGGGETALALLLLDRLNQLESKV 235
SEQ ID NO:6075  --------------APSREGSRGRR---------SDSGDDLIARAAKIIQDQ--------- 212
                                 .                       :

SEQ ID NO:6056  AKTGTPKPSRNQSPASSQTSAKSLARSQSSETKEQKHEMQKPRWKRQPNDDVTSNVTQCF 265
SEQ ID NO:6064  ----QQKPKQEKSDNSGKNTPK---KNKSRATSKERDLKDIPEWRRIPKG--ENSVAACF 300
SEQ ID NO:6060  ----RSKSKERSNSKTRDTTPK---------------NENKHTWKRTAGK---GDVTRFY 253
SEQ ID NO:6068  -----QQVTKQTAKEIRQKILN------------------KPRQKRSPNK--QCTVQQCF 286
SEQ ID NO:6071  -----KQVTKQSAKEVRQKILN------------------KPRQKRTPNK--QCPVQQCF 287
SEQ ID NO:6051  SGKGQQQQGQTVTKKSAAEASK------------------KPRQKRTATK--QYNVTQAF 275
SEQ ID NO:6075  -----QKKGSRITKAKADEMAH------------------RRYCKRTIPP--NYRVDQVF 247
                      :          :                        :*        *   :

SEQ ID NO:6056  GPRDLDH---NFGSAGVVANGVKAKGYPQFAELVPSTAAMLFDSHIVSKESG-------- 314
SEQ ID NO:6064  GPRGGFK---NFGDAEFVEKGVDASGYAQIASLAPNVAALLFGGNVAVRELA-------- 349
SEQ ID NO:6060  GARSSSA---NFGDTDLVANGSSAKHYPQLAECVPSVSSILFGSYWTSKEDG-------- 302
SEQ ID NO:6068  GKRGPNQ---NFGGGEMLKLGTSDPQFPILAELAPTAGAFFFGSRLELAKVQNLSGNLDE 343
SEQ ID NO:6071  GKRGPNQ---NFGGSEMLKLGTSDPQFPILAELAPTVGAFFFGSKLELVKKN--SGGADE 342
SEQ ID NO:6051  GRRGPEQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTP-------- 327
SEQ ID NO:6075  GPRTKGK-EGNFGDDKMNEEGIKDGRVTAMLNLVPSSHACLFGSRVTPKLQL-------- 298
                * *        ***.  .   *  .    . : . .*.  : :  .

SEQ ID NO:6056  ---NTVVLTFTTRVTVPKDHP----HLGKFLEELNAFTR------------------EMQ 349
SEQ ID NO:6064  ---DSYEITYNYKMTVPKSDP----NVELLVSQVDAFKTGNAK-LQRKKEKKNKRETTLQ 401
SEQ ID NO:6060  ---DQIEVTFTHKYHLPKDDP----KTGQFLQQINAYAR------------PSEVAKEQR 343
SEQ ID NO:6068  PQKDVYELRYNGAIRFDSTLSGFETIMKVLNENLNAYQQQD----GTMNMSPKPQRQ--R 397
SEQ ID NO:6071  PTKDVYELQYSGAVRFDSTLPGFETIMKVLNENLNAYQKDG----GADVVSPKPQRKGRR 398
SEQ ID NO:6051  ---SGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDAYKTFP----PTE------------ 368
SEQ ID NO:6075  ---DGLHLRFEFTTVVPCDDPQFDNYVKICDQCVDGVGTRPKDDEPKPKSRSSSRPATRG 355
                    .   : :     .     .           . ::.

SEQ ID NO:6056  QHPLLNPSALEFNPSQTSP-----ATAEPVRDEVSIETDIIDEVN-------------- 389
SEQ ID NO:6064  QHEEAIYDDVGAPSDVTHANLEWDTAVDGGDTAVEIINEIFDTGN-------------- 446
SEQ ID NO:6060  KRKSRSKSAERSEQDVVPDALIENYTDVFDDTQVEIIDEVTN----------------- 385
SEQ ID NO:6068  GQKNGQGENDNISVAAPKSRVQQNKIRELTAEDISLLKKMDEP-----FTEDTSEI--- 448
SEQ ID NO:6071  QAQEKKDEVDNVSVAKPKSSVQRNVSRELTPEDRSLLAQILDDGVVPDGLEDDSNV--- 454
```

```
SEQ ID NO:6051  -PKKDKKKKTDEAQPLPQRQKKQPTVTLLPAA----------------------------- 399
SEQ ID NO:6075  NSPAPRQQRPKKEKKLKKQDDEADKALTSDEERNNAQLEFYDEPKVINWGDAALGENEL 414
```

FIGURE 7E

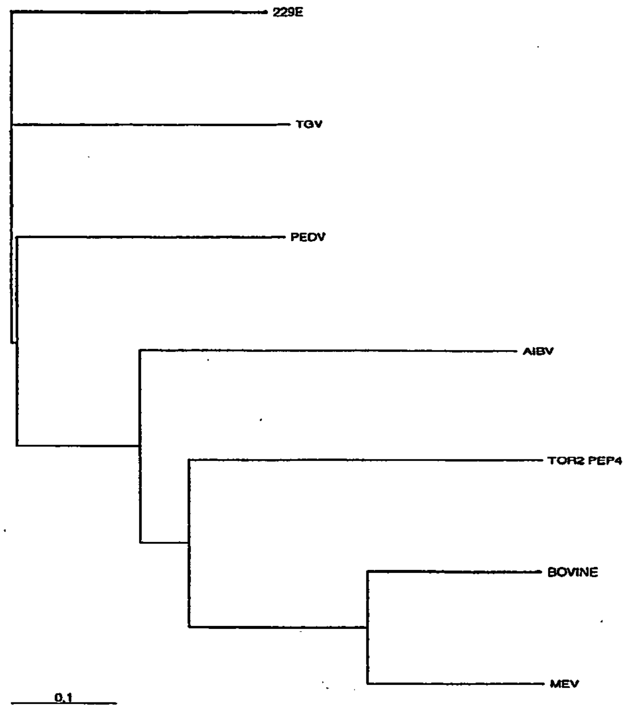


FIGURE 7F

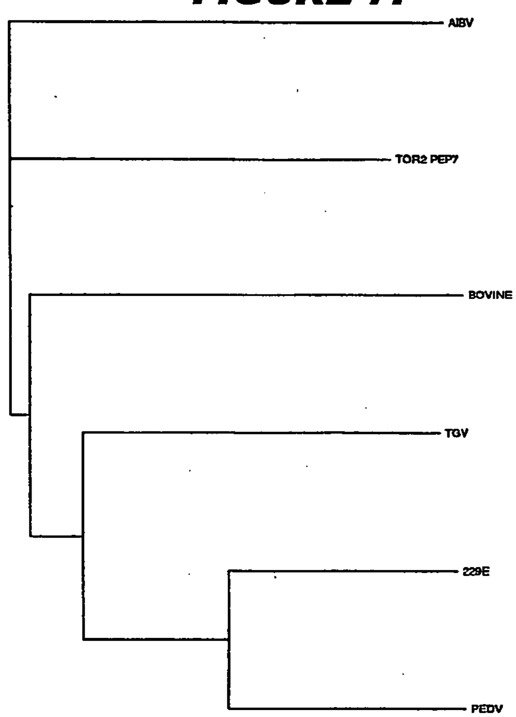


FIGURE 7G

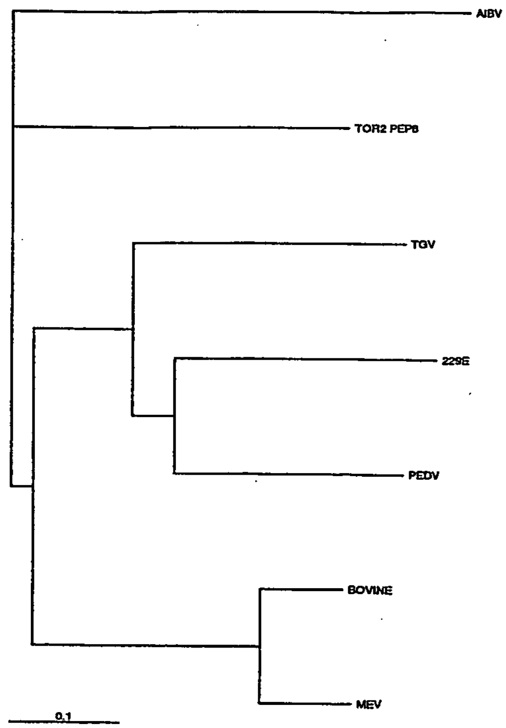


FIGURE 7H

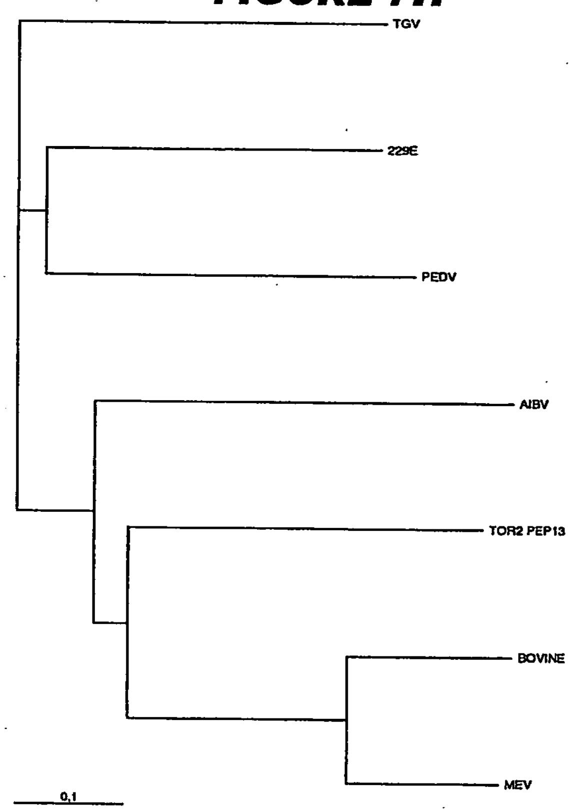


FIGURE 8

```
                                                                  Section 1
                              (1) 1         10         20         30          42
avian IBV partial 5'UTR 161-  (1) TATTAAAATCTTATTGTTGCTGGTATCACTGCTTGTTTTGCC
              HCoV-OC43 5'UTR (1) ------------------------------------------
              bovine CV 5'UTR (1) ------------------------------------------
                    Consensus (1)
                                                                  Section 2
                             (43) 43     50         60         70           84
avian IBV partial 5'UTR 161- (43) GTGTCTCACTTTATACATCTGTTGCTTGGGCTACCTAGTGTC
              HCoV-OC43 5'UTR (1) ------------------------------------------
              bovine CV 5'UTR (1) ------------------------------------------
                    Consensus (43)
                                                                  Section 3
                             (85) 85   90        100        110           126
avian IBV partial 5'UTR 161- (85) CAGCGTCCTACGGGCGTCGTGGCTGGTTCGAGTGCGAGGAAC
              HCoV-OC43 5'UTR (1) ------------------------------------------
              bovine CV 5'UTR (1) ------------------------------------------
                    Consensus (85)
                                                                  Section 4
                            (127) 127         140        150              168
avian IBV partial 5'UTR 161-(127) CTCTGGTTCATCTAGCGGTAGGCGGGTGTCTGGAAGTAGCAC
              HCoV-OC43 5'UTR (1) ---------GATTGTGAGCGATTTGCGTGCGTGCA--TCCCGC
              bovine CV 5'UTR (1) ---------GATTGCGAGCGATTTGCGTGCGTGCA--TCCCGC
                   Consensus (127)         GATTG GAGCGATTTGCGTGCGTGCA  TCCCGC
                                                                  Section 5
                            (169) 169        180        190        200      210
avian IBV partial 5'UTR 161-(169) TTCAGACGTACCGGTTCTGTTGTGTGAAATA--CGGGGTCAC
              HCoV-OC43 5'UTR (33) TTCA-------CTGATCTCTTGTTAGATCTTTTTCGTAATCTA
              bovine CV 5'UTR (33) TTCA-------CTGATCTCTTGTTAGATCTTTTCATAATCTA
                   Consensus (169) TTCA       CTGATCTCTTGTTAGATCTTTTCGTAATCTA
                                                                  Section 6
                            (211) 211       220        230        240       252
avian IBV partial 5'UTR 161-(209) CTCCCCCCACATACCTCTAAGGGCTTTTGAGCCTAGCGTTGG
              HCoV-OC43 5'UTR (68) AACTTTATAAAAACATCGACTCGCTGTAATCTATGCTTGTGG
              bovine CV 5'UTR (68) AACTTTATAAAAACATCCACTCCCTGTAGTCTATGCCTGTGG
                   Consensus (211) AACTTTATAAAAACATCCACTCCCTGTAGTCTATGCCTGTGG
                                                                  Section 7
                            (253) 253     260        270        280       294
avian IBV partial 5'UTR 161-(251) GCTACGTTCTCGCATAAGGTCGGCTATACGACGTTTGTAGGG
             HCoV-OC43 5'UTR (110) GCGTAGATTTTTCATAGTGGTGTTTATATT-CATTTCT-GCT
             bovine CV 5'UTR (110) GCGTAGATTTTTCATAGTGGTGTCTATATT-CATTTCT-GCT
                   Consensus (253) GCGTAGATTTTTCATAGTGGTGTCTATATT CATTTCT GCT
                                                                  Section 8
                            (295) 295  300         310        320          336
avian IBV partial 5'UTR 161-(293) GGTAGTGCCAAACAAGCCCTGAGGTGACAGGTTCTGGTGGTG
             HCoV-OC43 5'UTR (150) GTTAACAGCTTTCAGCCAGGGACGTGTTGTATCCTAGGC---
             bovine CV 5'UTR (150) GTTAACAGCTTTCAGCCAGGGACGTGTTGTATCCTAGGC---
                   Consensus (295) GTTAACAGCTTTCAGCCAGGGACGTGTTGTATCCTAGGC
                                                                  Section 9
                            (337) 337          350        360         373
            SEQ ID NO: 9910 (335) TTTAGTGAGCAGACATACAATAGACAGTGACAACATG
            SEQ ID NO: 9919 (189) ---AGTG-GCCCGCCCATAGGTCACAATG--------
            SEQ ID NO: 9892 (189) ---AGTG-GCCCACCCATAGGTCACAATG--------
                  Consensus (337)    AGTG GCCCACCCATAGGTCACAATG
```

FIGURE 9

		SEQ ID NO:
F1: $\mathrm{AT\frac{CTT}{TGC}G\frac{C}{A}G\frac{GT}{CG}A\frac{GGC}{TTT}G\frac{G}{C}GTG}$	(136-154 nt)	6021
F2: $\mathrm{GTG\frac{T}{C}GTG\frac{G}{C}AT\frac{AG}{CC}C\frac{A}{G}CTTCA}$	(152-172 nt)	6022
F3: $\mathrm{CTTCAC\frac{G}{T}G\frac{T}{A}TCT\frac{G}{C}TTGT\frac{GT}{TA}GA}$	(168-195nt)	6023
R1: $\mathrm{AG\frac{A}{G}A\frac{CCTGT}{TACAA}CAC\frac{C}{G}TC\frac{AGG}{CCT}GG\frac{T}{C}TG}$	(307-329nt)	6024
R2: $\mathrm{AAA\frac{C}{T}G\frac{CG}{AA}TATA\frac{GC}{AA}C\frac{GA}{AC}C\frac{CT}{AC}TATG}$	(265-288nt)	6025
R3: $\mathrm{C\frac{GA}{AC}C\frac{CT}{AC}TATG\frac{CG}{AA}A\frac{G}{A}A\frac{A}{T}C\frac{GTA}{TAC}GCCCA}$	(250-274nt)	6026

FIGURE 10

```
                                                                              Section 1
                                          (1) 1        10        20              36
avian IBV 3'UTR (NC_001451) 27103-        (1) GTAACATAATGGACCTGTTGTTTCCTGGTACATTTT
HCoV-OC43 3'UTR partial                   (1) ------------------------------------
bovine CV 3'UTR                           (1) ------------------------------------
Consensus                                 (1)
                                                                              Section 2
                                         (37) 37           50        60              72
avian IBV 3'UTR (NC_001451) 27103-       (37) GTTAAACACTATTTCTGTGCTTTCCTATCAATTATT
HCoV-OC43 3'UTR partial                   (1) ------------------------------------
bovine CV 3'UTR                           (1) ------------------------------------
Consensus                                (37)
                                                                              Section 3
                                         (73) 73     80        90                   108
avian IBV 3'UTR (NC_001451) 27103-       (73) ACAGGCATTGATTGTGATTATGTTCAATACTTAAGC
HCoV-OC43 3'UTR partial                   (1) ------------------------------------
bovine CV 3'UTR                           (1) ------------------------------------
Consensus                                (73)
                                                                              Section 4
                                        (109) 109        120       130              144
avian IBV 3'UTR (NC_001451) 27103-      (109) TTCTTTTGGTTGCTTTTTGCTTATTGTATTGTTGCT
HCoV-OC43 3'UTR partial                   (1) ------------------------------------
bovine CV 3'UTR                           (1) ------------------------------------
Consensus                               (109)
                                                                              Section 5
                                        (145) 145  150        160        170        180
avian IBV 3'UTR (NC_001451) 27103-      (145) GTGCTTTTTATTGTTGTGATTCTCATTAGTTTG--C
HCoV-OC43 3'UTR partial                   (1) -----------------------TAAGAGAATGAAC
bovine CV 3'UTR                           (1) --------------------------GAGAATGAAC
Consensus                               (145)                         A GAGAATGAAC
                                                                              Section 6
                                        (181) 181      190       200                216
avian IBV 3'UTR (NC_001451) 27103-      (179) TTTATCGTAGAAATTCAATAGTAAGAGTTAAGGAAG
HCoV-OC43 3'UTR partial                  (14) CTTAT-GTCGGCACCTGGTGGTAACCCCTC-GCAGG
bovine CV 3'UTR                          (11) CTTAT-GTCGGCACCTGGTGGTAACCCCTC-GCAGG
Consensus                               (181) CTTAT GTCGGCACCTGGTGGTAACCCCTC GCAGG
                                                                              Section 7
                                        (217) 217          230       240            252
avian IBV 3'UTR (NC_001451) 27103-      (215) ATAGGCATGTAGCTTGATTACCTACATGTCTATCGC
HCoV-OC43 3'UTR partial                  (48) AAAGTCGGG-------ATAAGGCAC-TCTCTATCAG
bovine CV 3'UTR                          (45) AAAGTCGGG-------ATAAGGCAC-TCTCTATCAG
Consensus                               (217) AAAGTCGGG       ATAAGGCAC TCTCTATCAG
```

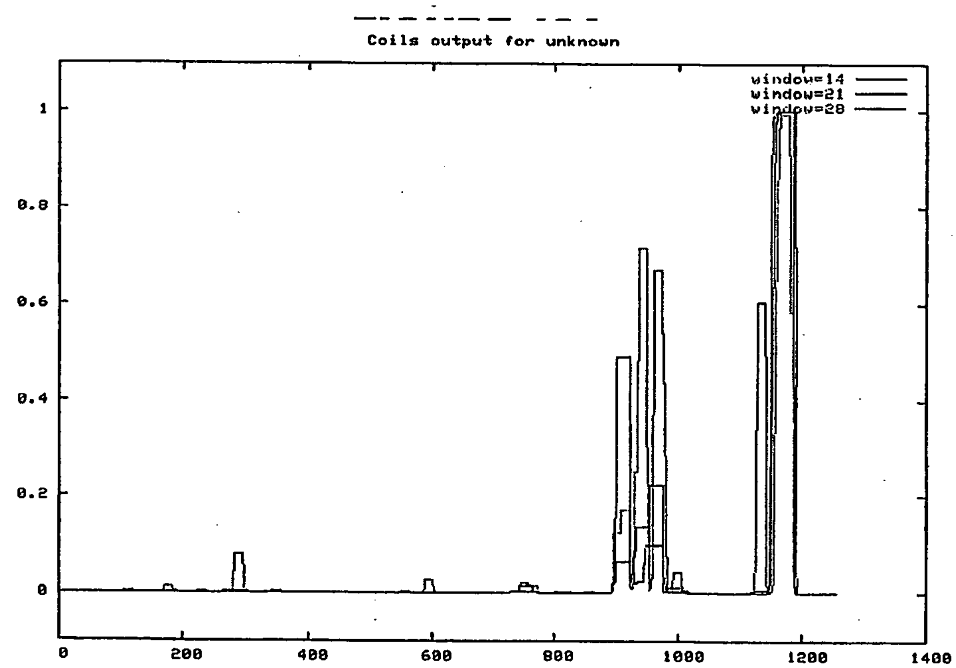
FIGURE 10 (contd.)

```
                                                                        Section 8
                                    (253) 253    260                270              288
avian IBV 3'UTR (NC_001451) 27103- (251) CAGGGAAATGTCTAATCTGTCTACTTAGTAGCCTGG
            HCoV-OC43 3'UTR partial  (76) AATGGA--TGTCTTGCTGCTATAATAGATAGA---G
                     bovine CV 3'UTR  (73) AATGGA--TGTCTTGCTGCTATAATAGATAGA---G
                           Consensus (253) AATGGA  TGTCTTGCTGCTATAATAGATAGA   G
                                                                        Section 9
                                    (289) 289              300        310        324
avian IBV 3'UTR (NC_001451) 27103- (287) AAACGAACGGTAGACCCTTAGATTTTAATTTAGTTT
            HCoV-OC43 3'UTR partial (107) AAGGTTATAGCAGACTAT-AGATT---AATTAGTTG
                     bovine CV 3'UTR (104) AAGGTTATAGCAGACTAT-AGATT---AATTAGTTG
                           Consensus (289) AAGGTTATAGCAGACTAT AGATT   AATTAGTTG
                                                                        Section 10
                                    (325) 325  330          340          350      360
avian IBV 3'UTR (NC_001451) 27103- (323) AATTTTTAGTTTAGTTTAAGTTAGT-TTAGAGTAGG
            HCoV-OC43 3'UTR partial (139) AAAGTTTTGTGTGGTAATGTATAGTGTTGGAGAAAG
                     bovine CV 3'UTR (136) AAAGTTTTGTGTGGTAATGTATAGTGTTGGAGAAAG
                           Consensus (325) AAAGTTTTGTGTGGTAATGTATAGTGTTGGAGAAAG
                                                                        Section 11
                                    (361) 361          370          380          396
avian IBV 3'UTR (NC_001451) 27103- (358) TATAAAGATGCCAGTGCCGGGGCCAC-GCGGAGTAC
            HCoV-OC43 3'UTR partial (175) TG-AAAGACT----TGCGGAAGTAATTGCCGACAAG
                     bovine CV 3'UTR (172) TG-AAAGACT----TGCGGAAGTAATTGCCGACAAG
                           Consensus (361) TG AAAGACT    TGCGGAAGTAATTGCCGACAAG
                                                                        Section 12
                                    (397) 397                410        420      432
avian IBV 3'UTR (NC_001451) 27103- (393) GATGGAGGGTACAGCAGTAGGAGGCCCATTAGGGGA
            HCoV-OC43 3'UTR partial (206) TGCCCAAAGGGAAGAGCCAGCACG----TTAAGTTA
                     bovine CV 3'UTR (203) TGCGCAAGGGGAAGAGCCAGCATG----TTAAGTTA
                           Consensus (397) TGCCCAAGGGGAAGAGCCAGCACG    TTAAGTTA
                                                                        Section 13
                                    (433) 433    440          450                468
avian IBV 3'UTR (NC_001451) 27103- (429) AGAGCTAAATTTTAGT--TTAAGTTAAGTTTAAT-T
            HCoV-OC43 3'UTR partial (238) CCACCCAGTAATTAGTAAATGAATGAAGTTAATTAT
                     bovine CV 3'UTR (235) CCATCCAGTAATTAGTAAATGAATGAAGTTAATTAT
                           Consensus (433) CCA CCAGTAATTAGTAAATGAATGAAGTTAATTAT
                                                                        Section 14
                                    (469) 469          480          490          504
avian IBV 3'UTR (NC_001451) 27103- (462) GGCTAAGTATAGTTAAAATTTATAGGCTAGTATAGA
            HCoV-OC43 3'UTR partial (274) GGCCAATTGGAAGAATCAC-----------------
                     bovine CV 3'UTR (271) GGCCAATTGGAAGAATCAC-----------------
                           Consensus (469)
                                                                        Section 15
                                    (505) 505     513
avian IBV 3'UTR (NC_001451) 27103- (498) GTTAGAGCA      SEQ ID NO: 9911
            HCoV-OC43 3'UTR partial (293) ---------      SEQ ID NO: 9920
                     bovine CV 3'UTR (290) ---------      SEQ ID NO: 9893
                           Consensus (505)
```

FIGURE 11

			SEQ ID NO:
F-1	$\mathrm{TCTATC}\frac{\mathrm{GCC}}{\mathrm{AGA}}\mathrm{A}\frac{\mathrm{G}}{\mathrm{T}}\mathrm{GGATGTCT}$	(245 ~ 265 nt)	6027
F-2	$\mathrm{TTAGTT}\frac{\mathrm{T}}{\mathrm{G}}\mathrm{AA}\frac{\mathrm{TT}}{\mathrm{AG}}\mathrm{TTT}\frac{\mathrm{A}}{\mathrm{T}}\mathrm{GT}\frac{\mathrm{T}}{\mathrm{G}}\mathrm{T}\frac{\mathrm{A}}{\mathrm{G}}\mathrm{GT}$	(318 ~ 339 nt)	6028
F-3	$\mathrm{TAGTGTT}\frac{\mathrm{A}}{\mathrm{G}}\mathrm{GAG}\frac{\mathrm{T}}{\mathrm{A}}\mathrm{A}\frac{\mathrm{G}}{\mathrm{A}}\mathrm{GT}\frac{\mathrm{A}}{\mathrm{G}}\mathrm{TAAAGA}$	(346 ~ 368 nt)	6029
R-1	$\mathrm{A}\frac{\mathrm{A}}{\mathrm{C}}\mathrm{TT}\frac{\mathrm{G}}{\mathrm{A}}\mathrm{GCCATA}\frac{\mathrm{A}}{\mathrm{T}}\mathrm{T}\frac{\mathrm{T}}{\mathrm{A}}\mathrm{AACTT}$	(458 ~ 476 nt)	6030
R-2	$\mathrm{ACTAA}\frac{\mathrm{TTAC}}{\mathrm{AATT}}\mathrm{T}\frac{\mathrm{G}}{\mathrm{A}}\mathrm{G}\frac{\mathrm{C}}{\mathrm{T}}\mathrm{T}\frac{\mathrm{GG}}{\mathrm{CT}}\mathrm{T}\frac{\mathrm{AA}}{\mathrm{CC}}\mathrm{C}\frac{\mathrm{T}}{\mathrm{C}}\mathrm{TAA}$	(426 ~ 448 nt)	6031
R-3	$\mathrm{T}\frac{\mathrm{TG}}{\mathrm{AC}}\mathrm{TC}\frac{\mathrm{G}}{\mathrm{C}}\mathrm{GC}\frac{\mathrm{AA}}{\mathrm{G}}\mathrm{T}\frac{\mathrm{TA}}{\mathrm{GG}}\mathrm{C}\frac{\mathrm{TT}}{\mathrm{CC}}\mathrm{C}\frac{\mathrm{C}}{\mathrm{G}}\mathrm{GCA}$	(375 ~ 395 nt)	6032 6033

FIGURE 12



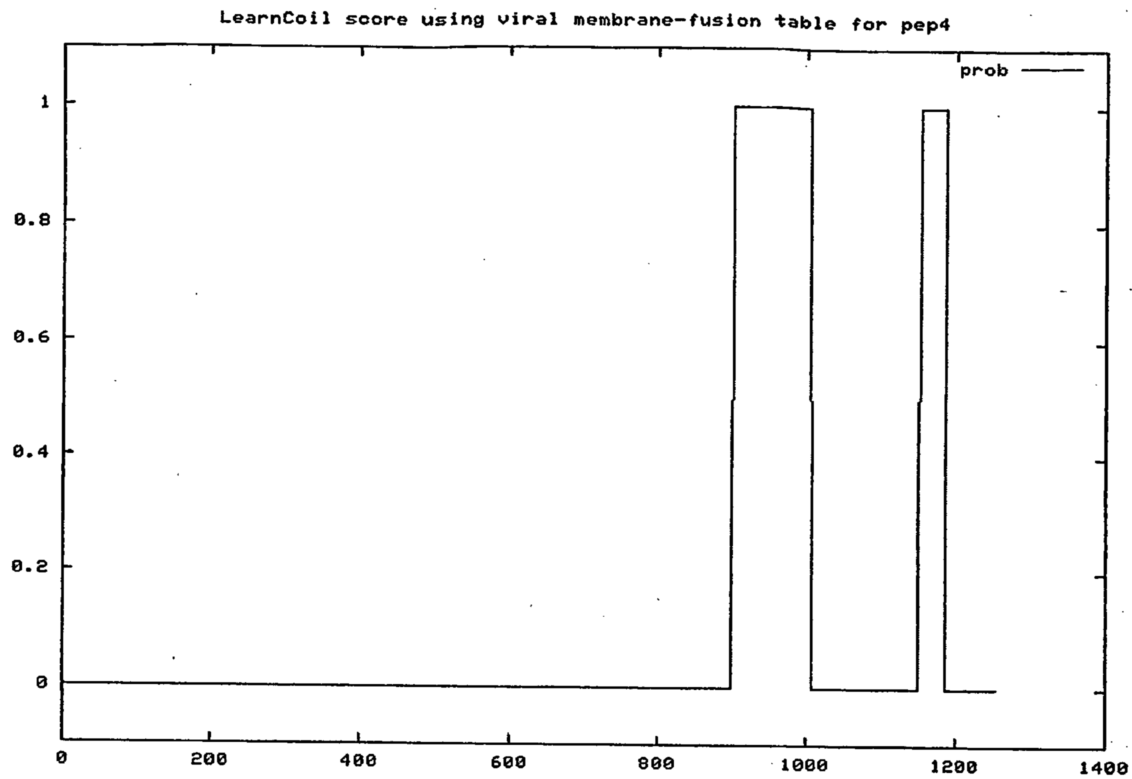

LearnCoil score using viral membrane-fusion table for pep4
prob
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400

FIGURE 13

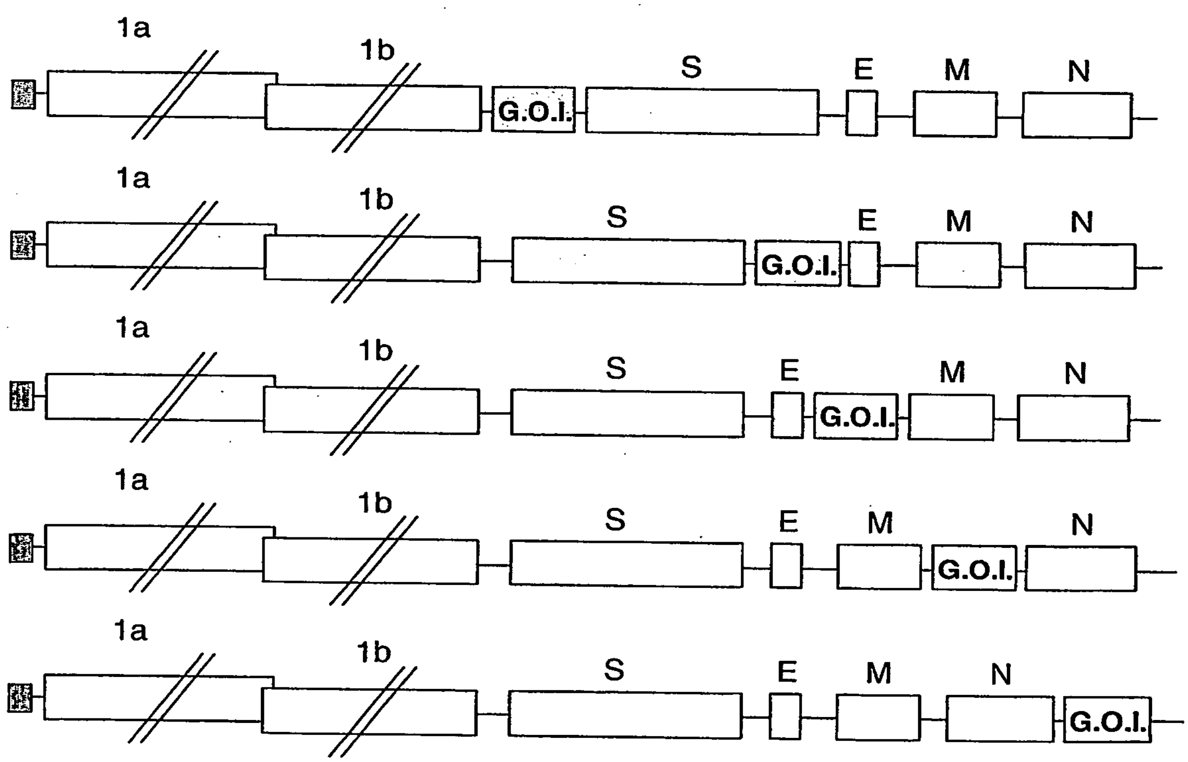

1a
1b
G.O.I.
S
E
M
N

FIGURE 14

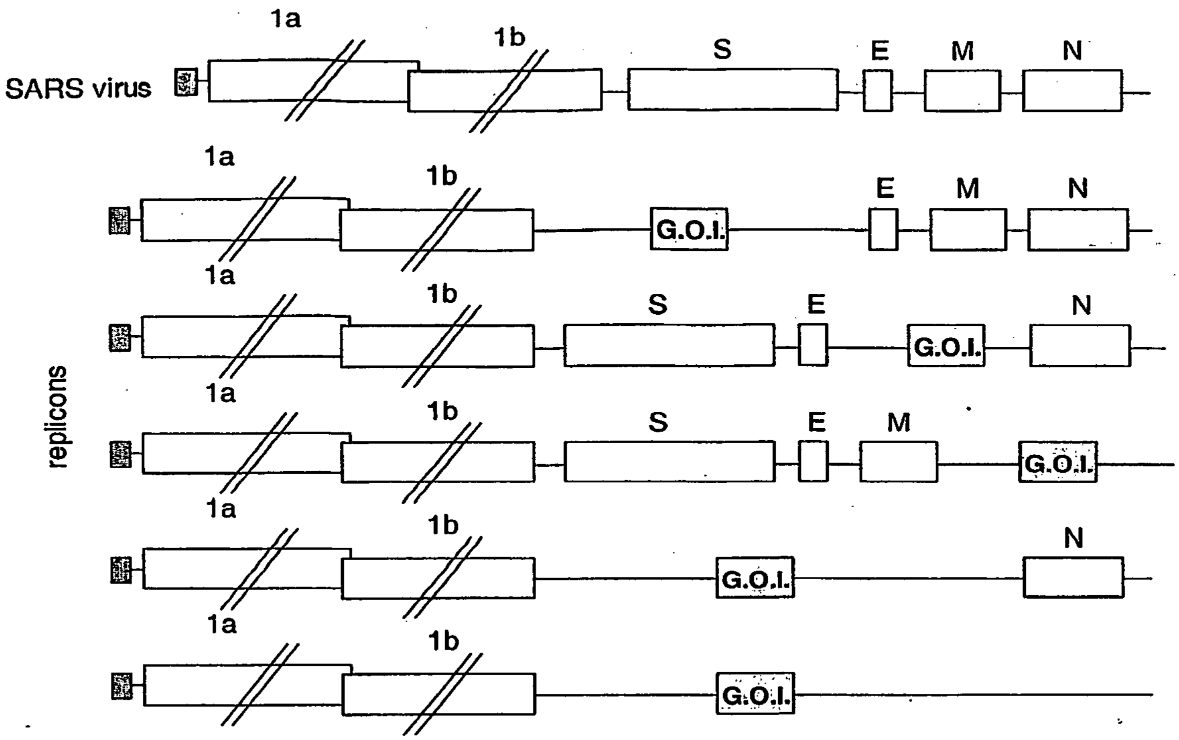


FIGURE 15

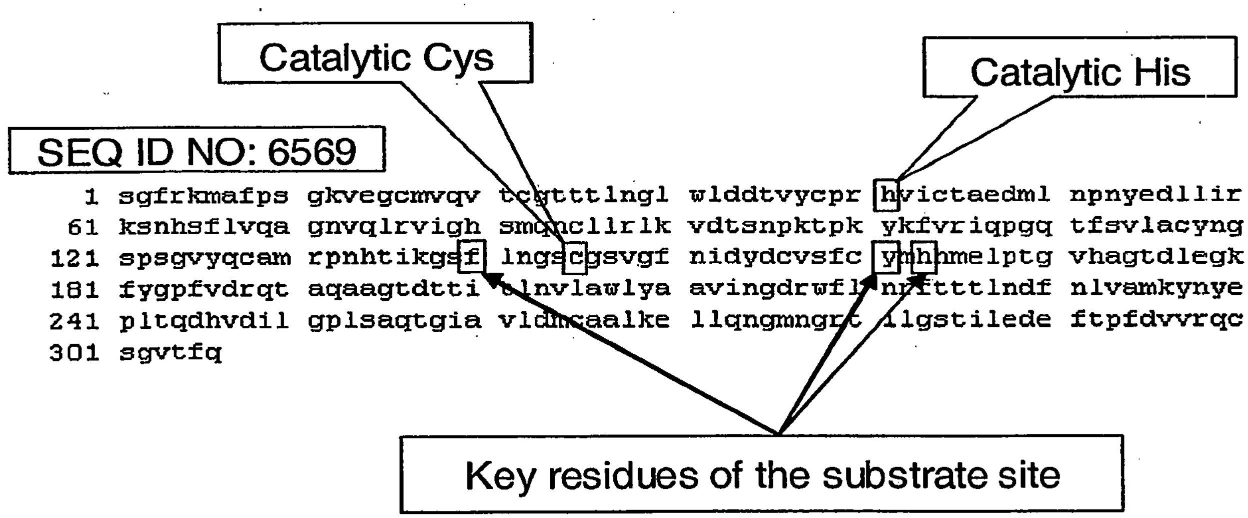


FIGURE 16

```
                                                                  Section 1
                (1)  1         10                  20                  30                  40         51
 avian IBV nsp2 (1)  SGFKKLVSPSSAVEKCIVSVSYRGNNLNGLWLGDSIYCPRHVLG---KFSG
       MHV nsp2 (1)  SGIVKMVSPTSKVEPCIVSVTYGNMTLNGLWLDDKVYCPRHVICSSADMTD
      SARS nsp2 (1)  SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDTVYCPRHVICTAEDMLN
      BCoV nsp2 (1)  SGIVKMVNPTSKVEPCIVSVTYGNMTLNGLWLDDKVYCPRHVICSASDMTN
      Consensus (1)  SGIVKMVSPSSKVEPCIVSVTYGNMTLNGLWLDDTVYCPRHVICSAADMTN
                                                                  Section 2
               (52)  52      60                70                  80                  90        102
 avian IBV nsp2 (49) DQHNDSLNLANNHEFEVTTQHGVTLNVVSRRLKGAVLILQTAMANAETPKY
       MHV nsp2 (52) PDYPNLLCRVTSSDFCVMSGR-MSLTVMSYQMQGCQLVLTVTLQNPNTPKY
      SARS nsp2 (52) PNYEDLLIRKSNHSFLVQAGN-VQLRVIGHSMQNCLLRLKVDTSNPKTPKY
      BCoV nsp2 (52) PDYTNLLCRVTSSDFTVLFDR-MSLTVMSYQMQGCMLVLTVTLQNSKTPKY
     Consensus (52)  PDY NLLCRVTSSDF VLSGR VSLTVMSYQMQGCLLVLTVTLQNPKTPKY
                                                                  Section 3
              (103)  103     110               120                 130                 140       153
 avian IBV nsp2 (100) KFIKANCGDSFTIACAYGGTVVGLYPVTMRSNGTIRASFLAGACGSVGFNV
   MHV nsp2 (102)    SFGVVKPGETFTVLAAYNGRPQGAFHVTLRSSHTIKGSFLCGSCGSVGYVL
  SARS nsp2 (102)    KFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNHTIKGSFLNGSCGSVGFNI
  BCoV nsp2 (102)    TFGVVKPGETFTVLAAYNGKPQGAFHVTMRSSYTIKGSFLCGSCGSVGYVL
 Consensus (103)     KFGVVKPGETFTVLAAYNGSPQGAFHVTMRSSHTIKGSFLCGSCGSVGFVI
                                                                  Section 4
              (154)  154     160               170                 180                 190       204
 avian IBV nsp2 (151) SKGVVNFFYMHHLELPNALHTGTDLMGEFYGGYVDEEVAQRVPPDNLVTNN
   MHV nsp2 (153)    TGDSVRFVYMHQLELSTGCHTGTDFSGNFYGPYRDAQVVQLPVQDYTQTVN
  SARS nsp2 (153)    DYDCVSFCYMHHMELPTGVHAGTDLEGKFYGPFVDRQTAQAAGTDTTITLN
  BCoV nsp2 (153)    MGDCVKFVYMHQLELSTGCHTGTDFNGDFYGPYKDAQVVQLPVQDYIQSVN
 Consensus (154)     DGDCVKFVYMHQLELSTGCHTGTDL GDFYGPYVDAQVVQLPVQDYTQTVN
                                                                  Section 5
              (205)  205   210                 220                 230                 240       255
 avian IBV nsp2 (202) IVAWLYAAIISVKESSFSLPKWLESTTVSVDDYNKWAGDNGFTPFSTS--T
   MHV nsp2 (204)    VVAWLYAAIFNRCN-------WFVQSDSCSLEEFNVWAMTNGFSSIKAD--L
  SARS nsp2 (204)    VLAWLYAAVINGDR-------WFLNRFTTTLNDFNLVAMKYNYEPLTQDHVD
  BCoV nsp2 (204)    FVAWLYAAIINNCN-------WFVQSDKCSVEDFNVWALSNGFSQVKSD--L
 Consensus (205)     VVAWLYAAIIN CN       WFLQSDTCSLEDFNVWAMSNGFSPIKSD  L
                                                                  Section 6
              (256)  256                270                 280                 290                306
 avian IBV nsp2 (251) ADTKLSAIIGVDVCKLLRTIMVKNS-QWGGDPILGQYNFSDELTPESVRNQ
   MHV nsp2 (247)    VEDALASMTGVTVEQVLAAIKRLHS-GFQGKQILGSCVLEDELTPSDVYQQ
  SARS nsp2 (249)    ILGPLSAQTGIAVLDMCAALKELLQNGMNGRTILGSTILEDEFTPFDVVRQ
  BCoV nsp2 (247)    VIDALASMTGVSLETLLAAIKRLKN-GFQGRQIMGSCSFEDELTPSDVYQQ
 Consensus (256)     VIDALAAMTGVSVE LLAAIKRL S GFQGRQILGSCILEDELTPSDVYQQ
                                                                  Section 7
              (307)  307   313
 avian IBV nsp2 (301) IGGVRLQ     SEQ ID NO: 6570
   MHV nsp2 (297)    LACVKLQ     SEQ ID NO: 6571
  SARS nsp2 (300)    CSGVTFQ     SEQ ID NO: 6569
  BCoV nsp2 (297)    LAGIKLQ     SEQ ID NO: 6572
 Consensus (307)     LAGVKLQ     SEQ ID NO: 6573
```

FIGURE 17

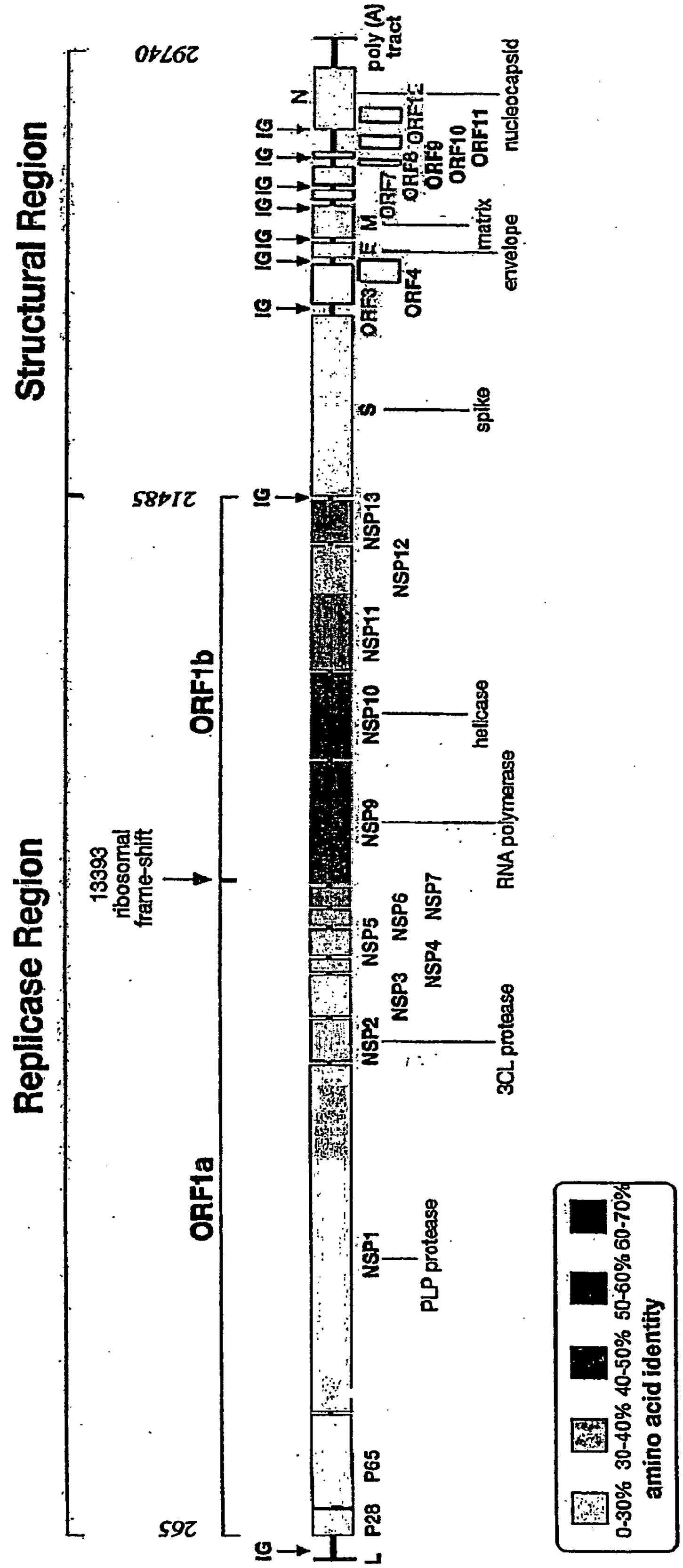

Structural Region
Replicase Region
ORF1a
ORF1b
265
21485
29740
13393
ribosomal
frame-shift
IG
L
P28
P65
NSP1
PLP protease
NSP2
NSP3
NSP4
NSP5
NSP6
NSP7
3CL protease
NSP9
RNA polymerase
NSP10
helicase
NSP11
NSP12
NSP13
S
spike
ORF3
ORF4
E
envelope
M
matrix
ORF7
ORF8
ORF9
ORF10
ORF11
ORF12
N
nucleocapsid
poly (A)
tract
0-30%
30-40%
40-50%
50-60%
60-70%
amino acid identity

FIGURE 18

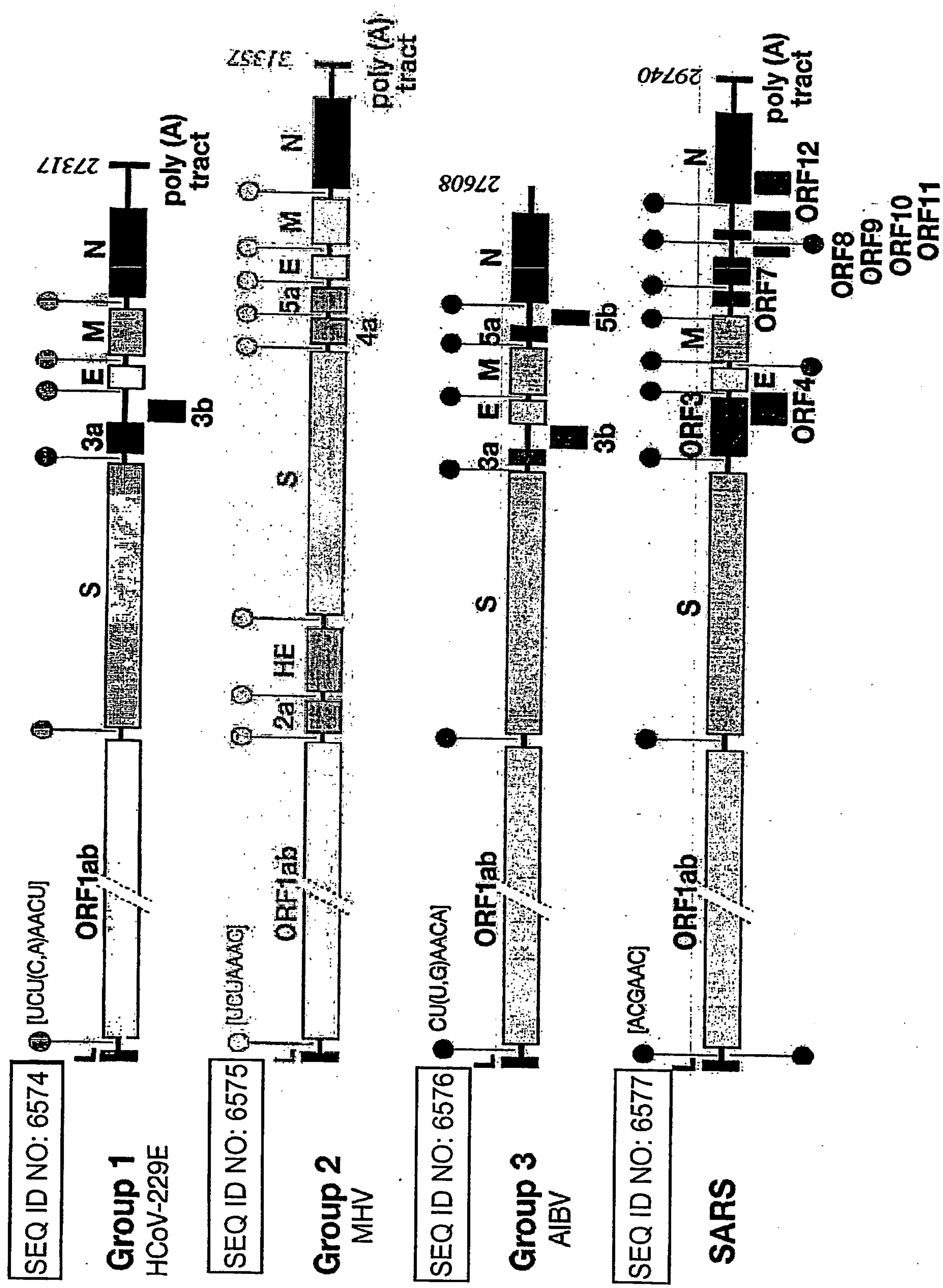

SEQ ID NO: 6574
Group 1
HCoV-229E
[UCU(C,A)AACU]
L
ORF1ab
S
3a
3b
E
M
N
27317
poly (A) tract
SEQ ID NO: 6575
Group 2
MHV
[UCUAAAC]
L
ORF1ab
2a
HE
S
4a
5a
E
M
N
31357
poly (A) tract
SEQ ID NO: 6576
Group 3
AIBV
CU(U,G)AACA]
L
ORF1ab
S
3a
3b
E
M
5a
5b
N
27608
SEQ ID NO: 6577
SARS
[ACGAAC]
L
ORF1ab
S
ORF3
ORF4
E
M
ORF7
ORF8
ORF9
ORF10
ORF11
ORF12
N
29740
poly (A) tract

FIGURE 19

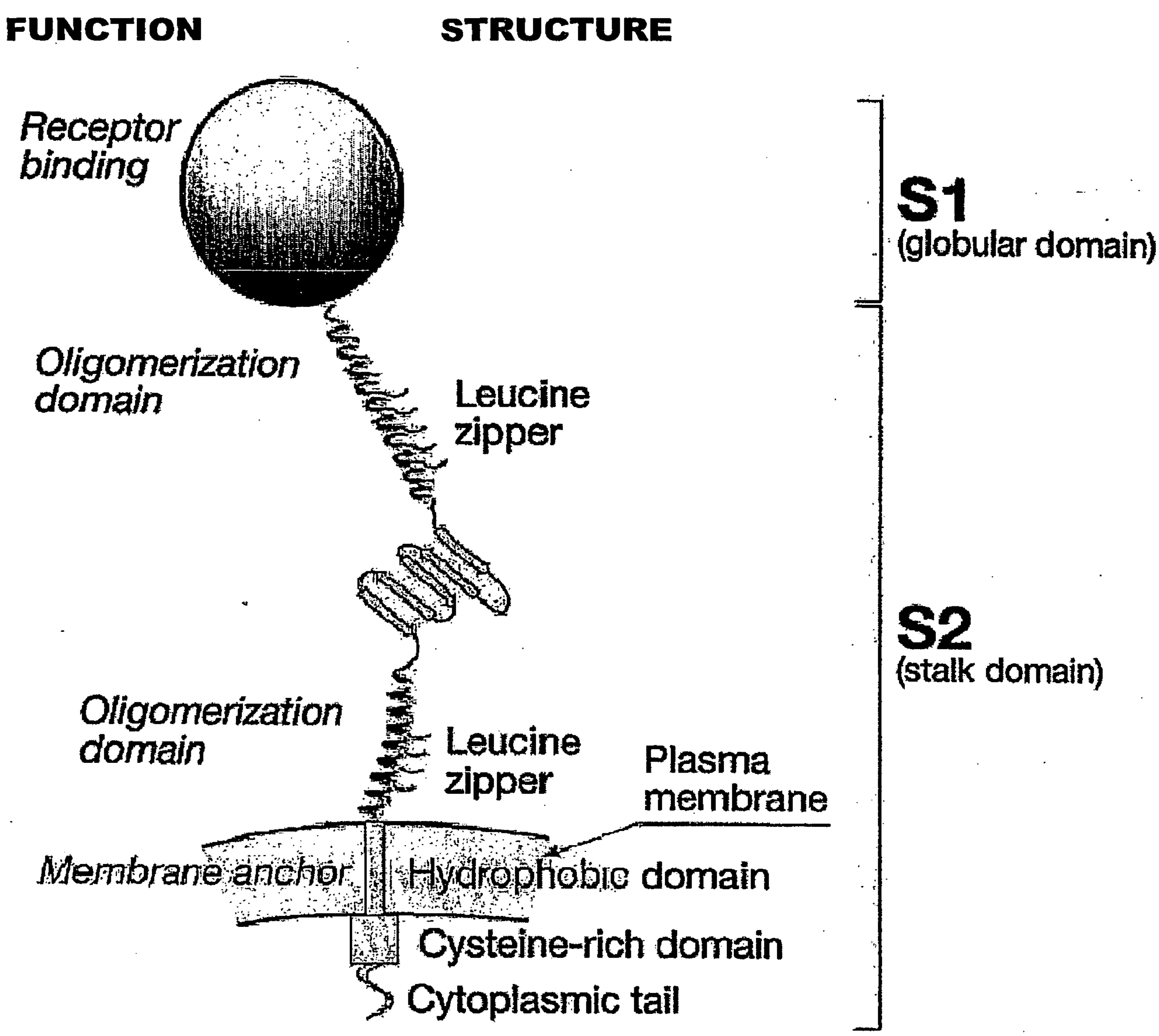

FUNCTION
STRUCTURE
Receptor binding
S1
(globular domain)
Oligomerization domain
Leucine zipper
S2
(stalk domain)
Oligomerization domain
Leucine zipper
Plasma membrane
Membrane anchor
Hydrophobic domain
Cysteine-rich domain
Cytoplasmic tail

FIGURE 20

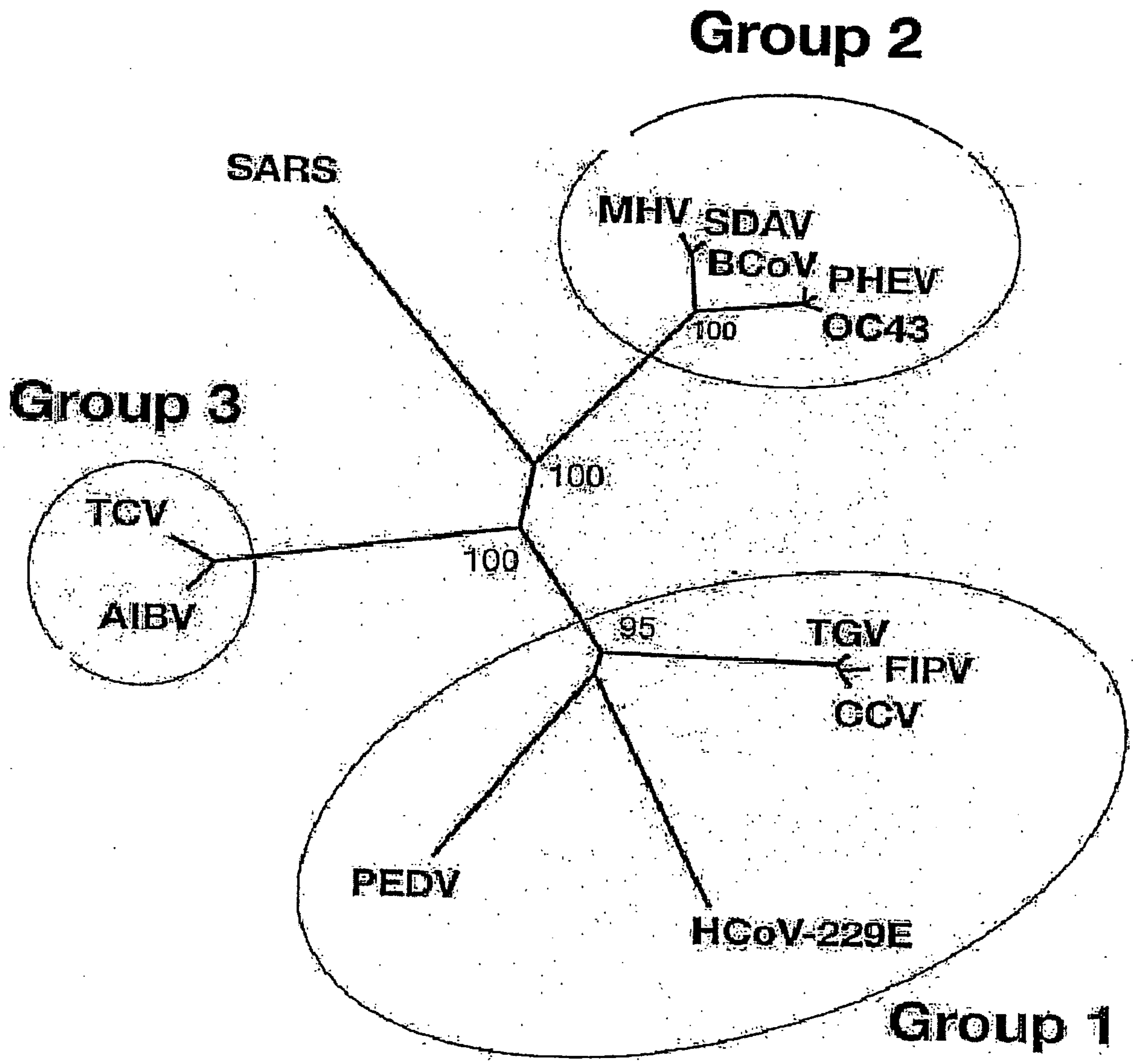

Group 2
SARS
MHV
SDAV
BCoV
PHEV
OC43
100
Group 3
100
TCV
100
AIBV
95
TGV
FIPV
CCV
PEDV
HCoV-229E
Group 1

FIGURE 21

FIGURE 21A

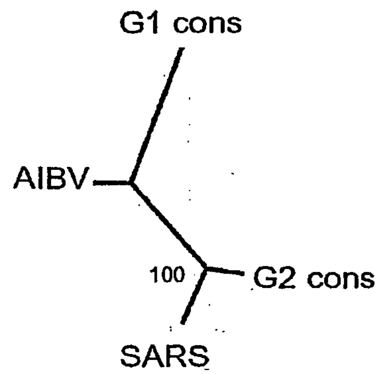


FIGURE 21B

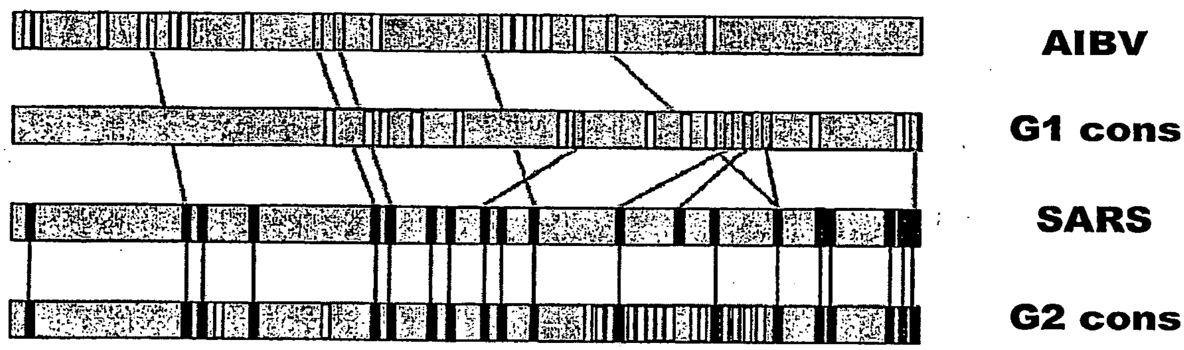


FIGURE 22

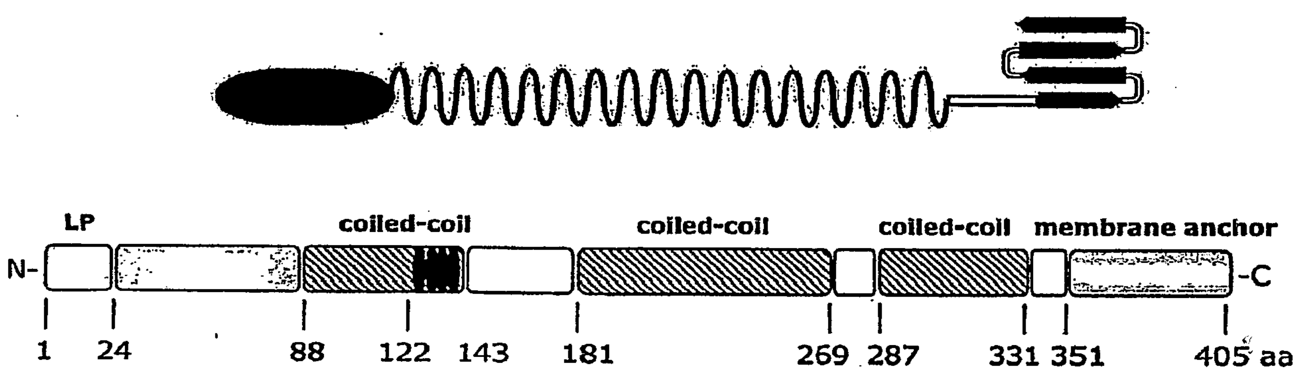


FIGURE 23

LPRKSQPTSISCRSVL-TNFKICVAVARLHA-CTYAV-TIINFTVVDKKRVTRPSSADCL
RFRPCCSRSSAYLGFVRV-PKGKMESLVLGVNEKTHVQLSLPVLQVRDVLVRGFGDSVEE
ALSEAREHLKNGTCGLVELEKGVLPQLEQPYVFIKRSDALSTNHGHKVVELVAEMDGIQY
GRSGITLGVLVPHVGETPIAYRNVLLRKNGNKGAGGHSYGIDLKSYDLGDELGTDPIEDY
EQNWNTKHGSGALRELTRELNGGAVTRYVDNNFCGPDGYPLDCIKDFLARAGKSMCTLSE
QLDYIESKRGVYCCRDHEHEIAWFTERSDKSYEHQTPFEIKSAKKFDTFKGECPKFVFPL
NSKVKVIQPRVEKKKTEGFMGRIRSVYPVASPQECNNMHLSTLMKCNHCDEVSWQTCDFL
KATCEHCGTENLVIEGPTTCGYLPTNAVVKMPCPACQDPEIGPEHSVADYHNHSNIETRL
RKGGRTRCFGGCVFAYVGCYNKRAYWVPRASADIGSGHTGITGDNVETLNEDLLEILSRE
RVNINIVGDFHLNEEVAIILASFSASTSAFIDTIKSLDYKSFKTIVESCGNYKVTKGKPV
KGAWNIGQQRSVLTPLCGFPSQAAGVIRSIFARTLDAANHSIPDLQRAAVTILDGISEQS
LRLVDAMVYTSDLLTNSVIIMAYVTGGLVQQTSQWLSNLLGTTVEKLRPIFEWIEAKLSA
GVEFLKDAWEILKFLITGVFDIVKGQIQVASDNIKDCVKCFIDVVNKALEMCIDQVTIAG
AKLRSLNLGEVFIAQSKGLYRQCIRGKEQLQLLMPLKAPKEVTFLEGDSHDTVLTSEEVV
LKNGELEALETPVDSFTNGAIVGTPVCVNGLMLLEIKDKEQYCALSPGLLATNNVFRLKG
GAPIKGVTFGEDTVWEVQGYKNVRITFELDERVDKVLNEKCSVYTVESGTEVTEFACVVA
EAVVKTLQPVSDLLTNMGIDLDEWSVATFYLFDDAGEENFSSRMYCSFYPPDEEEEDDAE
CEEEEIDETCEHEYGTEDDYQGLPLEFGASAETVRVEEEEEEDWLDDTTEQSEIEPEPEP
TPEEPVNQFTGYLKLTDNVAIKCVDIVKEAQSANPMVIVNAANIHLKHGGGVAGALNKAT
NGAMQKESDDYIKLNGPLTVGGSCLLSGHNLAKKCLHVVGPNLNAGEDIQLLKAAYENFN
SQDILLAPLLSAGIFGAKPLQSLQVCVQTVRTQVYIAVNDKALYEQVVMDYLDNLKPRVE
APKQEEPPNTEDSKTEEKSVVQKPVDVKPKIKACIDEVTTTLEETKFLTNKLLLFADING
KLYHDSQNMLRGEDMSFLEKDAPYMVGDVITSGDITCVVIPSKKAGGTTEMLSRALKKVP
VDEYITTYPGQGCAGYTLEEAKTALKKCKSAFYVLPSEAPNAKEEILGTVSWNLREMLAH
AEETRKLMPICMDVRAIMATIQRKYKGIKIQEGIVDYGVRFFFYTSKEPVASIITKLNSL
NEPLVTMPIGYVTHGFNLEEAARCMRSLKAPAVVSVSSPDAVTTYNGYLTSSSKTSEEHF
VETVSLAGSYRDWSYSGQRTELGVEFLKRGDKIVYHTLESPVEFHLDGEVLSLDKLKSLL
SLREVKTIKVFTTVDNTNLHTQLVDMSMTYGQQFGPTYLDGADVTKIKPHVNHEGKTFFV
LPSDDTLRSEAFEYYHTLDESFLGRYMSALNHTKKWKFPQVGGLTSIKWADNNCYLSSVL
LALQQLEVKFNAPALQEAYYRARAGDAANFCALILAYSNKTVGELGDVRETMTHLLQHAN
LESAKRVLNVVCKHCGQKTTTLTGVEAVMYMGTLSYDNLKTGVSIPCVCGRDATQYLVQQ
ESSFVMMSAPPAEYKLQQGTFLCANEYTGNYQCGHYTHITAKETLYRIDGAHLTKMSEYK
GPVTDVFYKETSYTTTIKPVSYKLDGVTYTEIEPKLDGYYKKDNAYYTEQPIDLVPTQPL
PNASFDNFKLTCSNTKFADDLNQMTGFTKPASRELSVTFFPDLNGDVVAIDYRHYSASFK
KGAKLLHKPIVWHINQATTKTTFKPNTWCLRCLWSTKPVDTSNSFEVLAVEDTQGMDNLA
CESQQPTSEEVVENPTIQKEVIECDVKTTEVVGNVILKPSDEGVKVTQELGHEDLMAAYV
ENTSITIKKPNELSLALGLKTIATHGIAAINSVPWSKILAYVKPFLGQAAITTSNCAKRL
AQRVFNNYMPYVFTLLFQLCTFTKSTNSRIRASLPTTIAKNSVKSVAKLCLDAGINYVKS
PKFSKLFTIAMWLLLLSICLGSLICVTAAFGVLLSNFGAPSYCNGVRELYLNSSNVTTMD
FCEGSFPCSICLSGLDSLDSYPALETIQVTISSYKLDLTILGLAAEWVLAYMLFTKFFYL
LGLSAIMQVFFGYFASHFISNSWLMWFIISIVQMAPVSAMVRMYIFFASFYYIWKSYVHI
MDGCTSSTCMMCYKRNRATRVECTTIVNGMKRSFYVYANGGRGFCKTHNWNCLNCDTFCT
GSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVAVKNGALHLYFDKAGQKTYERHPLSH
FVNLDNLRANNTKGSLPINVIVFDGKSKCDESASKSASVYYSQLMCQPILLLDQALVSDV
GDSTEVSVKMFDAYVDTFSATFSVPMEKLKALVATAHSELAKGVALDGVLSTFVSAARQG
VVDTDVDTKDVIECLKLSHHSDLEVTGDSCNNFMLTYNKVENMTPRDLGACIDCNARHIN
AQVAKSHNVSLIWNVKDYMSLSEQLRKQIRSAAKKNNIPFRLTCATTRQVVNVITTKISL
KGGKIVSTCFKLMLKATLLCVLAALVCYIVMPVHTLSIHDGYTNEIIGYKAIQDGVTRDI
ISTDDCFANKHAGFDAWFSQRGGSYKNDKSCPVVAAIITREIGFIVPGLPGTVLRAINGD
FLHFLPRVFSAVGNICYTPSKLIEYSDFATSACVLAAECTIFKDAMGKPVPYCYDTNLLE
GSISYSELRPDTRYVLMDGSIIQFPNTYLEGSVRVVTTFDAEYCRHGTCERSEVGICLST
SGRWVLNNEHYRALSGVFCGVDAMNLIANIFTPLVQPVGALDVSASVVAGGIIAILVTCA
AYYFMKFRRVFGEYNHVVAANALLFLMSFTILCLVPAYSFLPGVYSVFYLYLTFYFTNDV
SFLAHLQWFAMFSPIVPFWITAIYVFCISLKHCHWFFNNYLRKRVMFNGVTFSTFEEAAL

CTFLLNKEMYLKLRSETLLPLTQYNRYLALYNKYKYFSGALDTTSYREAACCHLAKALND
FSNSGADVLYQPPQTSITSAVLQSGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDTVY
CPRHVICTAEDMLNPNYEDLLIRKSNHSFLVQAGNVQLRVIGHSMQNCLLRLKVDTSNPK
TPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNHTIKGSFLNGSCGSVGFNIDYDCV
SFCYMHHMELPTGVHAGTDLEGKFYGPFVDRQTAQAAGTDTTITLNVLAWLYAAVINGDR
WFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMCAALKELLQNGMN
GRTILGSTILEDEFTPFDVVRQCSGVTFQGKFKKIVKGTHHWMLLTFLTSLLILVQSTQW
SLFFFVYENAFLPFTLGIMAIAACAMLLVKHKHAFLCLFLLPSLATVAYFNMVYMPASWV
MRIMTWLELADTSLSGYRLKDCVMYASALVLLILMTARTVYDDAARRVWTLMNVITLVYK
VYYGNALDQAISMWALVISVTSNYSGVVTTIMFLARAIVFVCVEYYPLLFITGNTLQCIM
LVYCFLGYCCCCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQGLLPPKSSIDAFKL
NIKLLGIGGKPCIKVATVQSKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILL
AKDTTEAFEKMVSLLSVLLSMQGAVDINRLCEEMLDNRATLQAIASEFSSLPSYAAYATA
QEAYEQAVANGDSEVVLKKLKKSLNVAKSEFDRDAAMQRKLEKMADQAMTQMYKQARSED
KRAKVTSAMQTMLFTMLRKLDNDALNNIINNARDGCVPLNIIPLTTAAKLMVVVPDYGTY
KNTCDGNTFTYASALWEIQQVVDADSKIVQLSEINMDNSPNLAWPLIVTALRANSAVKLQ
NNELSPVALRQMSCAAGTTQTACTDDNALAYYNNSKGGRFVLALLSDHQDLKWARFPKSD
GTGTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNNLNRGMVLGSLAATVRLQAGNATEV
PANSTVLSFCAFAVDPAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTPEANMDQES
FGGASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPVGFTLRNTVCTVCGMWKGYG
CSCDQLREPLMQSADASTFLNGFAV-VQPVLHRAAQALVLMSSTGLLIFTTKKLLVLQSS
-KLIAVASRRRMRKAIY-TLTL-LRGILCLTTNMKRLFITWLKIVQRLLSMTFSSLE-MV
TWYHIYHVSV-LNTQWLI-SMLYVILMRVIVIH-KKYSSHTIAVMMIISIRRIGMTS-RI
LTSYAYMLT-VSVYANHY-RLYNSAMLCVMQAL-AY-H-IIRILMGTGTISVISYK-HQA
AEFLLWIHITHC-CPSSL-LGHWLLSPIWMLISQNHLLSGIC-NMILRKRDFVSSTVILN
IGTRHTIPIVLTVWMIGVSFIVQTLMCYFLLCFHLQVLDH--EKYL-MVFLLLFQLDTIF
VS-ESYIIRM-TYIARVSVSRNF-CMLLIQLCMQLLAIYC-INALHAFQ-LH-QTMLLFK
LSNPVILIKTFMTLLCLKVSLRKEVLLN-NTSSLLRMATLLSVIMTIIVIICQQCVISDN
SYS-LKLLINTLIVTMVAVLMPTK-SLTIWINQLVSHLINGVRLDFIMTQ-VMRIKMHFS
RILSVMSSLL-LK-ILSMPLVQRIELAP-LVSLSVVL-QIDSFIRNY-SQ-PPLEELLW-
LEQASFTVAGIIC-KLFTVM-KLHTLWVGIIQNVTEPCLTCLG-WPLLFLLANITLAVTY
HTVSTG-LTSVRKY-VRWSCVAAHYMLNQVEHHPVMLQLLMLIVSLTFVKLLQPM-MHFF
QLMVIR-LTSMSAIYNTGSMSVSIEIGMLIMNSWMSFTLTCVNISP--FFLMMPLCAITV
TMRLKV--LALRTLRQFFIIKIMCSCLRQNVGLRLTLLKDLTNFAHSIQC-LNKEMITCT
CLTQIHQEY-AQAVLSMILSKQMVHL-LKGSCHWLLMLTHLQNILIRSMLMSFTCIYNTL
ESYMMSLLATCWTCIP-C-LMITPHGTGNLSFMRLCTHHIQSCRL-VLVYCAIHRLHFVA
VPVLGDHSYVASAAMTMSFQHHTN-CCLLIPMFAMPQVVMSLM-HNCI-EV-AIIASHIS
LPLVFHYVLMVRFLVYTKTHV-AVTMSLTSMR-QHVIGLMLAITYLPTLVLRDSSFSQQK
RSKPLRKHLSCHMVLPLYAKYSLTENCIFHGRLENLDHH-TETMSLLVTV-LKIVKYRLE
STPLKKVTMVMLLCTEVLRHTS-MLVITLC-HLTL-CHLVHLL-CHKSTM-ELLACTQHS
TSQMSFLAMLQIIKRSACKSTLHSKDHLVLVRVILPSDLLSITHLLA-CIRHALMQLLMP
YVKRH-NICP-INVVESYLRVRA-SVLINSK-IQH-NSMFSAL-MHCQKQLLTL-SLMKS
LWLLIMT-VLSMLDFVQNTTSILAILLNYQPPAHC-LKAH-NQNILIQCADL-KQ-VQTC
SLELVAVVLLKLLTL-VL-FMTIS-KHTRISQLNASKCSTKVLLHMMFHLQSTDLK-AL-
ENFLHAILLGEKLFLSHLIIHRTL-LQKS-DCLRRLLIHHRVLNMTMSYSHKLLKQHTLV
MSTASMWLSQGQKLAFCA-CLIEIFMTNCNLQV-KYHVAMWLHYKQKM-LDFLRTVVRSL
LVFILHRHLHTSALI-SSRLKDYVLTYQAYQRT-PTVDSSL-WVSK-ITKSMVTLICLSP
AKKLFVTFVRGLALM-RAVMQLEMLWVLTYLSS-DFLQVLT--LYRLVMLTLKITQNSPE
LMQNLHQVTSLNILYHSCIKACPGM-CVLR-YKCSVIH-KDCQTESCSSFGRMALSLHQ-
STLSRLDLKERVVCVTNVQLAFLLHQILMPAGIILWVLTMSITHL-LMFSSGALRVTFRV
TMTNIARYMEMHMWLVVMLS-LDV-QSMSALLSALIGLLNTLL-EMN-GLILLAEKYNTW
L-SLHCLLISFQFFMTLEIQRLSSVCLRLK-NGSSTMLSHVVTKLTK-RNSSILMLHITI
NSLMVFVCFGIVTLIVTQPMQLCVGLTQESCQT-TYQAVMVVVCM-ISMHSTLQLSIKVH
LLI-SNCLSFTILIVLVSLMANK-CRILIMFHSNLLRVLHDAI-VVLFADTMQMSTDSTW
MHII--FLLLDLAYGFTNNLILITCGIHLPGYRV-KMWLIMLLIKDTLMDTPAKHLFPSLI
MLFTQR-MVLMWRSLKIRQHFLLMLHLSFGLSVTLNQCQRLRYSIIWVLISLLLIL-SGTT

KEKPQHMYLQ-VSAQ-LTLPRNLLRVLVLHLLSCLMVEWKDR-TFLETPVMVF--QKVQS
KV-HLQRDQHKLASMESH-LENQ-KHSLTTLRK-TALFNSCLKPTLLRAET-RILSPDHK
WKLTFSSSLWMNSYSDISSRAMPSNTSFMEISVMDNLAVFI---A-PSAHKIHHLN-RIL
SLWTAQ-KITS-QMRKQVHQNVCVL-LIFYLMTLSR--SHKICQ-FQKWSRLQLTMLKFH
SCFGVRMDMLKPSTQNYKQVERGNQVLRCLTCTRCKECFLKSVTFRIMVKMLLYQKE---
MSQSILNCVNT-IHLL-LYPTT-ELFTLVLALIKELHQVQLCSDNGCQLAHYLSIQILMT
SSPTHILL-LETVQQYIRLINGTLLLAICMTLGPNM-QKRMTLKKGFSLICVDL-SKN-P
WVVL-L-R-QSILGMLTFTSLWAISHGGQLLLQM-MHHHRKHF-LGLTILASRRNKLMAI
PCMLTTFSGGTQILSSCLPIHSLT-ANFLLN-EELL-CLLRRIKSMI-FILFWKKVGLSL
EKTTELWFQVIFLLTTKRTCLFSYYFLLSLVVVTLTGAPLLMMFKLLITLNILHL-GGFT
ILMKFLDQTLFI-LRIYFFHFILMLQGFILLIIRLATLSYLLRMVFILLPQRNQMLSVVG
FLVLP-TTSHSR-LLLTILLMLLYEHVTLNCVTTLSLLFLNPWVHRHIL-YSIMHLIALS
STYLMPFRLMFQKSQVILNTYESLCLKIKMGFSMFIRAINL-M-FVIYLLVLTL-NLFLS
CLLVLTLQILEPFLQPFHLLKTFGARQLQPILLAI-SQLHLCSSMMKMVQSQMLLIVLKI
HLLNSNALLRALRLTKEFTRPLISGLFPQEML-DSLILQTCVLLERFLMLLNSLLSMHGR
EKKFLIVLLITLCSTTQHFFQPLSAMAFLPLS-MIFASPMSMQILL-SREMM-DK-RQDK
LVLLLIIIINCQMISWVVSLLGILGTLMLLQLVIIIINIGILDMASLGPLRETYLMCLSP
LMANLAPHLLLIVIGH-MIMVFTPLLALATNLTEL-YFLLNF-MHRPRFVDQNYPLTLLR
TSVSILILMDSLVLVC-LLLQRDFNHFNNLAVMFLISLIPFEILKHLKY-TFHLALLGV-
V-LHLEQMLHLKLLFYIKMLTALMFLQQFMQINSHQLGAYILLETMYSRLKQAVL-ELSM
STLLMSATFLLELAFVLVTIQFLYYVVLAKNLLWLILCL-VLIVQLLTLITPLLYLLTFQ
LALLQK-CLFLWLKPP-IVICTSAEILLNVLICFSNMVAFAHN-IVHSQVLLLNRIATHV
KCSLKSNKCTKPQL-NILVVLIFHKYYLTL-SQLRGLLLRTCSLIR-HSLMLAS-SNMAN
A-VILMLEISFVRRSSMDLQCCHLCSLMI-LLPTLLL-LVVLPLLDGHLVLALLFKYLLL
CKWHIGSMALELPKMFSMRTKNKSPTNLTRRLVKFKNHLQQHQLHWASCKTLLTRMLKH-
THLLNNLALILVQFQVC-MISFRDLIKSRRRYKLTG-LQADFKAFKPM-HNN-SGLLKSG
LLLILLLLKCLSVFLDNQKELTFVERATTLCPSHKQPRMVLSSYMSRMCHPRRGTSPQRQ
QFVMKAKHTSLVKVFLCLMALLGLLHRGTSFLHK-LLQTIHLSQEIVMSLLASLTTQFMI
LCNLSLTHSKKSWTSTSKIIHHQMLILATFQALTLLSSTFKKKLTASMRSLKI-MNHSLT
FKNWENMSNILNGLGMFGSASLLD-LPSSWLQSCFVA-LVVAVASRVHALVVLAASLMRM
TLSQFSRVSNYITHKRTYGFVYEIFYSWINYCTASKN-QCFSCKYCSCYSNDTATSLTPF
RMACYWRCISCCFSERYQNNCAQ-KMAASPL-GLPVHLQFTAAICYHLFTSFACRCRYGG
AIFVPLCLDIFSTMHQRM-NYYEMLALLEVQIQEPITL-CQLLCLLAHT-L-LLYTI-QC
HRYNCRY-R-RHFNTKTQRRLPNWWLF-G-ALRC-RLCRCTWLFHRSLLPA-VYTNYYRH
WY-KCYILHL-QAC-RPTECANTHNRRLFRSC-SSNGSNL--ADDDY-RAFVSTRK-VRT
YVLIRFGRNRYVNS--RTSFSCFRGILASHTSHPYCASIVCVLLQYC-REFSKTNGLRLL
AC-KSELF-RSS-SSGLNELTIIIIILFGTLTLLIMADNGTITVEELKQLLEQWNLVIGFL
FLAWIMLLQFAYSNRNRFLYIIKLVFLWLLWPVTLACFVLAAVYRINWVTGGIAIAMACI
VGLMWLSYFVASFRLFARTRSMWSFNPETNILLNVPLRGTIVTRPLMESELVIGAVIIRG
HLRMAGHSLGRCDIKDLPKEITVATSRTLSYYKLGASQRVGTDSGFAAYNRYRIGNYKLN
TDHAGSNDNIALLVQ-VTTDVSSC-LPGYNSRDIDYHYEDFQDCYLES-RYNKFNSETII
-ASN-EELFGVR--RTYGVRLSIKRT-KLFSS-H-LYLHLASYITIRSVLEVRLYY-KNL
AHQEHTRAIHHFTLLLTINLH-LALAHTLLLLVLTVLDIPISCVQDQFHQNFSSDKRRFN
KSSTRHFFSLLLL-YF-YFASPLRERQNE-AHFN-LLFVLFSLSAIPCFNNAYYILVFTR
NPGSRRTLYQSLNEHETSHCFDLYFSMQLHMHCSTALCI--TSCA-RSL-GTTLGVILIA
LLGFVL-ERFYLFIDGTLWFKHAHLMLLSTVKIQLVVRL-LGVGTFMKVTKLLHLETYLL
F-INEQIKMSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPNNTAS
WFTALTQHGKEELRFPRGQGVPINTNSGPDDQIGYYRRATRRVRGGDGKMKELSPRWYFY
YLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLPKGF
YAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRLNQLE
SKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAFGRRGPEQTQGNFGDQDL
IRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYHGAIKLDDKDPQFKDNVI
LLNKHIDAYKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTLLPAADMDDFSRQLQNSM
SGASADSTQA-TLMMTTQGRWAM-TFSQFRLRYIVYSCAE-ILVTKQHK-V-LTLISHSN
L-SMCNIREDLKEPPHFHRGHAEYDRGYSE-C-GELPIWKSPNV-N-F—CYPHVILIAS

FIGURE 24

TQEKPTNLDLL-ICSLNEL-NLCSCRSAACLVHLRSINNNKFYCR-QETSNSSLFCRLLT
VSSVLQSIISIPRFRPGVTER-DGEPCSWCQRENTRPTQFACPSG-RRASAWLRGLCGRG
PIGGT-TPQKWHLWSSRAGKRRTAPA-TALCVH-TF-CLKHQSRPQGR-AGCRNGRHSVR
S-RYNTGSTRATCGRNPNCIPQCSSS-ER--GSRWS-LWHRSKVL-LR-RAWH-SH-RL-
TKLEH-AWQWCTP-THS-AQWRCSHSLCRQQFLWPRWVPS-LHQRFSRTRGQVNVHSFRT
T-LHRVEERCLLLP-P-A-NCLVH-AL--ELRAPDTLRN-ECQEI-HFQRGMPKVCVSS-
LKSQSHSTTC-KEKD-GFHGAYTLCVPCCISTGV-QYALVYLDEM-SLR-SFMADVRLSE
SHL-TLWH-KFSY-RTYYMWVPTY-CCSENAMSCLSRPRDWT-A-CCRLSQPLKH-NSTP
QGR-D-MFWRLCVCLCWLL--ACLLGSSC-C-YWLRPYWHYW-QCGDLE-GSP-DTES-T
C-H-HCWRFSFE-RGCHHFGIFLCFYKCLY-HYKES-LQVFQNHC-VLR-L-SYQGKARK
RCLEHWTTEISFNTTVWFSLTGCWCYQINFCAHT-CSKPLNS-FAKSSCHHT-WYF-TVI
TSCRRHGLYFRPAHQQCHYYGICNWWSCTTDFSVVV-SFGHYC-KTQAYL-MD-GET-CR
S-ISQGCLGDSQISHYRCF-HRQGSNTGCFR-HQGLCKMLH-CC-QGTRNVH-SSHYRWR
KVAITQLR-SLHRSKQGTLPSVYTWQGAAATTHAS-GTKRSNLS-R-FT-HSTYL-GGCS
QER-TRSTRDAR--LHKWSYRRHTSLCKWPHALRD-GQRTILRIVSWFTGYKQCLSLKRG
CTN-RCNLWRRYCLGSSRLQECENHI-A--TC-QSA--KVLCLHC-IRYRSY-VCMCCSR
GCCEDFTTSF-SPYQHGY-S--VECSYILLI--CW-RKLFITYVLFLLPSR-GRRGRCRV
-GRRN--NL-T-VRYRG-LSRSPSGIWCLS-NSSS-GRRRGRLAG-YY-AIRD-ARTRTY
T-RTS-SVYWLFKTY-QCCH-MC-HR-GGTKC-SYGDCKCC-HTPETWWWCSRCTQQGNQ
WCHAKGE--LH-AKWPSYSRRVLFAFWT-SC-EVSACCWT-PKCR-GHPAS-GSI-KFQF
TGHLTCTIVVSRHIWC-TTSVFTSVRADGSYTGLYCSQ-QSSL-AGCHGLS--PEA-SGS
T-TRGATKHRRFQN-GEICRTEACRCEAKN-GLH--GYHNTGRN-VSYQ-VTLVC-YQW-
ALP-FSEHA-R-RYVFP-EGCTLHGR-CYH-W-YHLCCNTLQKGWWHY-DALKSFEESAS
--VYNHVPWTRMCWLYT-GS-DCS-EMQICILCTTFRST-C-GRDSRNCILEFERNACSC
-RDKKINAYMHGC-SHNGNHPT-V-RN-NSRGHR-LWCPILLLY--RACSFYYYEAELSK
-AACHNANWLCDTWF-S-RGCALYAFS-SSCRSVSIITRCCYYI-WIPHFVIKDI-GALC
RNSFFGWLLQRLVLFRTAYRVRC-IS-AW-QNCVPHSGEPRRVSS-R-GSFT-QTKESLI
PAGG-DYKSVHNCGQH-SPHTACGYVYDIWTAVWSNILGWC-CYKN-TSCKS-G-DFLCT
T---HTT--SFRVLPYS--EFSW-VHVCFKPHKEMEISSSWWFNFN-MG--QLLFV-CFI
STSTA-SQIQCTSTSRGLL-SPCW-CC-LLCTHTRLQ--NCWRAW-CQRNYDPSSTAC-F
GICKASS-CGV-TLWSENYYLNGCRSCDVYGYSIL--S-DRCFHSMCVWS-CYTISSTTR
VFFCYDVCTTC-V-ITARYILMCE-VHW-LSVWSLHSYNC-GDPLSY-RSSPYKDVRVQR
TSD-CFLQGNILHYNHQACVV-TRWSYLHRD-TKIGWVL-KG-CLLYRAAYRPCTNSTIT
KCEF--FQTHMF-HKIC--FKSNDRLHKASFTRAICHILPRLEWRCSGY-L-TLFSEFQE
RC-ITA-ANCLAH-PGYNQDNVQTKHLVFTLSLEYKASRYFKFI-SSGSRRHTRNGQSCL
-KSTTHL-RSSGKSYHTEGSHRV-RENYRSCRQCHT-TIR-RC-SNTRVRS-GSYGCLCG
KHKHYH-ET--AFTSLRFKNNCHSWYCCN--CSLE-NFGLCQTILRTSSNYNIKLR-EIS
TTCV-QLYALCVYIIVPIVYFY-KYQF-N-SFTTYNYC-K-C-ECC-IMFGCRH-LCEVT
QIF-IVHNRYVAIVVKYLLRFSNLCNCCFWCTLI-FWCSFLL-WR-RIVS-FV-RYYYGF
L-RFFSLQHLFKWIRLP-FLSSS-NHSGDDFIVQARLDNFRSGR-VGFGIYVVHKILLFI
RSFSYNAGVLWLFC-SFHQQFLAHVVYH-YCTNGTRFCNG-DVHLLCFFLLHMEELCSYH
GWLHLFDLHDVL-AQSCHTR-VYNYC-WHEEIFLCLCKWRPWLLQDSQLELSQL-HILHW
-YIH---SCS-FVTPV-KTNQPY-PVIVYC--CCCEKWRASPLL-QGWSKDL-ETSALPF
CQFRQFES-QH-RFTAY-CHSF-WQVQMRRVCF-VCFCVLQSADVPTYSVA-PSSCIRRW
R-Y-SFR-DV-CLCRHLFSNF-CSYGKT-GTCCYSSQRVSKGCSFRWCPFYIRVSCPTRC
C-YRC-HKGCY-MSQTFTSL-LRSDR-QL-QFHAHL--G-KHDAQRSWRMY-L-CKAYQC
PSSKKSQCFTHLECKRLHVFI-TAA-TNS-CCQEEQHTF-TNLCYN-TGCQCHNY-NLTQ
GW-DC-YLF-TYA-GHIIVRSCCIGLLYRYASTYIVNP-WLHK-NHWLQSHSGWCHS-HH
FY--LFCK-TCWF-RMV-PAWWFIQK-QKLPCSSCYHYKRDWFHSAWLTGYCAESNQW-L
LAFSTSCF-CCWQHLLHTFQTH-V--FCYLCLRSCC-VYNF-GCYGQTCAILL-H-FARG
FYFL--ASSRHSLCAYGWFHHTVS-HLPGGFC-SSNNF-C-VL-TWYMRKVRSRYLPIYQ
W-MGS---ALQSSIRSFLWC-CDESHS-HLYSSCATCGCFRCVCFSSGWWYYCHIGDLCC
LLLYEIQTCFW-VQPCCCC-CTFVFDVFHYTLSGTSLQLSAGSLLSLLLVLDILFHQ-CF
ILGSPSMVCHVFSYCAFLDNSNLCILYFSEALPLVL-QLS-EKSHV-WSYI-YLRGGCFV

YLFAQQGNVPKIA-RDTVATYTV-QVSCSI-QVQVFQWSLRYYQLS-SSLLPLSKGSK-L
-QLRC-CSLPTTTDINHFCCSAEWF-ENGIPVRQS-RVHGTSNLWNYNS-WIVVG-HSIL
SKTCHLHSRRHA-S-L-RSAHSQIQP-LSCSGWQCSTSCYWPFYAKLSA-A-S-YF-P-D
TQV-ICPYPTWSNIFSSSMLQWFTIWCLSVCHET-SYH-RFFP-WIMW-CWF-H-L-LRV
FLLYASYGASNRSTRWY-LRR-ILWSIC-QTNCTGCRYRHNHNIKCFGMAVCCCYQW--V
VS--IHHYFE-L-PCGNEVQL-TFDTRSC-HIGTSFCSNRNCRLRYVCCFERAAAEWYEW
SYYPW-HYFRR-VYTI-CC-TMLWCYLPR-VQENC-GHSSLDAFNFLDITIDSCSKYTVV
TVFLCLRECFLAIYSWYYGNCCMCYAAC-A-ARILVLVSVTFSCNSCLL-YGLHAC-LGD
AYHDMA-IG-H-LVWL-A-GLCYVCFSFSFAYSHDSSHCL--CC-TCLDTDECHYTCLQS
LLW-CFRSSYFHVGLSYFCNL-LFWCRYDYHVFS-SYSVCVC-VLPIVIYYWQHLTVYHA
CLLFLRLLLLLLLWPFLFTQPLLQAYSWCL-LLGLYTRI-VYELPGAFAS-E-Y-CFQA-
H-VVGYWR-TMYQGCYCTV-NV-RKVHICGTALGSSTT-SRVIF-IVGTMCTTPQ-YSSC
KRHN-SFREDGFSFVCFAIHAGCCRH--VVRGNAR-PCYSSGYCFRI-FFTIICRLCHCP
GGL-AGCS-W-F-SRSQKVKEIFECG-I-V-P-CCHATQVGKDGRSGYDPNVQTGKI-GQ
EGKSN-CYANNALHYA-EA---CT-QHYQQCA-WLCSTQHHTIDYSSQTHGCCP-LWYLQ
EHL-W-HLYICICTLGNPASC-CG-QDCST--N-HGQFTKFGLASYCYSSKSQLSC-TTE
--TESSSTTTDVLCGWYHTNSLY--QCTCLL-QFEGR-VCAGITIRPPRSQMG-IP-E-W
YRYNLHRTGTTL-VCYRHTKRA-SEILVLHQRLKQPK-RYGAGQFSCYSTSSGWKCYRST
CQFNCAFLLCFCSRPC-SI-GLPSKWRTTNHQLCEDVVYTHWYRTGNYCNTRS-HGPRVL
WWCFMLSVL-MPH-PSKS-RIL-LER-VRPNTYHLC--PSGFYT-KHSLYRLRNVERLWL
-L-PTPRTLDAVCGCINVFKRVCGVSAARLTPCGTGTSTDVVYRAFDIYNEKVAGFAKFL
KTNCCRFQEKDEEGNLLDSYFVVKRHTMSNYQHEETIYNLVKDCPAVAVHDFFKFRVDGD
MVPHISRQRLTKYTMADLVYALRHFDEGNCDTLKEILVTYNCCDDDYFNKKDWYDFVENP
DILRVYANLGERVRQSLLKTVQFCDAMRDAGIVGVLTLDNQDLNGNWYDFGDFVQVAPGC
GVPIVDSYYSLLMPILTLTRALAAESHMDADLAKPLIKWDLLKYDFTEERLCLFDRYFKY
WDQTYHPNCINCLDDRCILHCANFNVLFSTVFPPTSFGPLVRKIFVDGVPFVVSTGYHFR
ELGVVHNQDVNLHSSRLSFKELLVYAADPAMHAASGNLLLDKRTTCFSVAALTNNVAFQT
VKPGNFNKDFYDFAVSKGFFKEGSSVELKHFFFAQDGNAAISDYDYYRYNLPTMCDIRQL
LFVVEVVDKYFDCYDGGCINANQVIVNNLDKSAGFPFNKWGKARLYYDSMSYEDQDALFA
YTKRNVIPTITQMNLKYAISAKNRARTVAGVSICSTMTNRQFHQKLLKSIAATRGATVVI
GTSKFYGGWHNMLKTVYSDVETPHLMGWDYPKCDRAMPNMLRIMASLVLARKHNTCCNLS
HRFYRLANECAQVLSEMVMCGGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVNALLS
TDGNKIADKYVRNLQHRLYECLYRNRDVDHEFVDEFYAYLRKHFSMMILSDDAVVCYNSN
YAAQGLVASIKNFKAVLYYQNNVFMSEAKCWTETDLTKGPHEFCSQHTMLVKQGDDYVYL
PYPDPSRILGAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTKHPNQEYADVFHLYLQYIR
KLHDELTGHMLDMYSVMLTNDNTSRYWEPEFYEAMYTPHTVLQAVGACVLCNSQTSLRCG
ACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKP
PISFPLCANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLKLFAAET
LKATEETFKLSYGIATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQIGE
YTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQEHYVRITGLYPTLN
ISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDAL
CEKALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEIS
MATNYDLSVVNARLRAKHYVYIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMF
LGTCRRCPAEIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAINRPQIGVVR
EFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCN
VNRFNVAITRAKIGILCIMSDRDLYDKLQFTSLEIPRRNVATLQAENVTGLFKDCSKIIT
GLHPTQAPTHLSVDIKFKTEGLCVDIPGIPKDMTYRRLISMMGFKMNYQVNGYPNMFITR
EEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQLGFSTGVNLVAVPTGYVDTENNTEFTRV
NAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMK
YFVKIGPERTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGFTGNLQSN
HDQHCQVHGNAHVASCDAIMTRCLAVHECFVKRVDWSVEYPIIGDELRVNSACRKVQHMV
VKSALLADKFPVLHDIGNPKAIKCVPQAEVEWKFYDAQPCSDKAYKIEELFYSYATHHDK
FTDGVCLFWNCNVDRYPANAIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSAF
TNLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKSATCITRCNLGGAVCRHHANEYRQYLD
AYNMMISAGFSLWIYKQFDTYNLWNTFTRLQSLENVAYNVVNKGHFDGHAGEAPVSIINN
AVYTKVDGIDVEIFENKTTLPVNVAFELWAKRNIKPVPEIKILNNLGVDIAANTVIWDYK

REAPAHVSTIGVCTMTDIAKKPTESACSSLTVLFDGRVEGQVDLFRNARNGVLITEGSVK
GLTPSKGPAQASVNGVTLIGESVKTQFNYFKKVDGIIQQLPETYFTQSRDLEDFKPRSQM
ETDFLELAMDEFIQRYKLEGYAFEHIVYGDFSHGQLGGLHLMIGLAKRSQDSPLKLEDFI
PMDSTVKNYFITDAQTGSSKCVCSVIDLLLDDFVEIIKSQDLSVISKVVKVTIDYAEISF
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VAKYTQLCQYLNTLTLAVPYNMRVIHFGAGSDKGVAPGTAVLRQWLPTGTLLVDSDLNDF
VSDAYSTLIGDCATVHTANKWDLIISDMYDPRTKHVTKENDSKEGFFTYLCGFIKQKLAL
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PDEIFRSDTLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWV
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LAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWER
KKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQT
GVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSP
DGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKN
QCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVS
VITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHV
DTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSI
SITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDRNTRE
VFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC
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QMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALN
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SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPA
ICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDP
LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
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RPRR-QRQYCFASTVSDNRCFILLTSRLQ-QRY-LSL-GLSGLLFGILTL--VQ--DNYL
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ELYSPLFLIVAALVFLILCFTIKRKTE-MSSL-LTSICAF-PFCYSLF--CLLYFGFHSK
SRI-KNLVPKSKRT-NFSLF-LVFLYAVAYAL-YSAVHLINLMCLKILVRYNTRGNTYST
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LNKRTN-NV--WTPIKPT-CPPHYIWWTHRFN-Q-PEWRTQWGKAKTAPTPRFTQ-YCVL
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WSFC-FNSGINTHDDHTRQMGYVNVFAIPFTIHSLLLCRMNSRN-TAQVGLVNFNLT-QS
LINV-H-GGLERATTFSSRPRGVRSRVQ-IMLGRAAYMEEP-CVKLILVVLSPCDFNSFL

FIGURE 25

PTPRTLDAVCGCINVFKRVCGVSAARLTPCGTGTSTDVVYRAFDIYNEKVAGFAKFLKTNCCRFQE
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FIGURE 26

FIGURE 26A

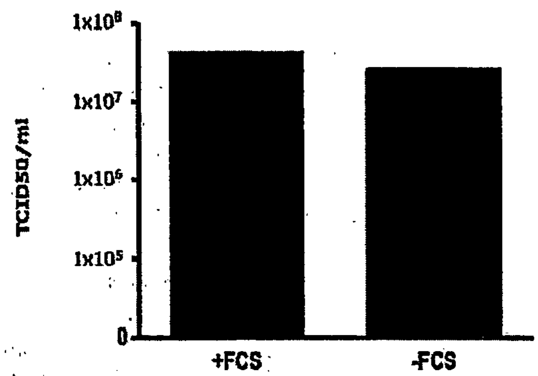


FIGURE 26B

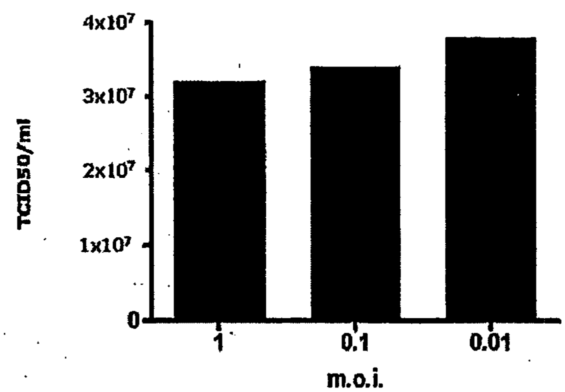


FIGURE 26C

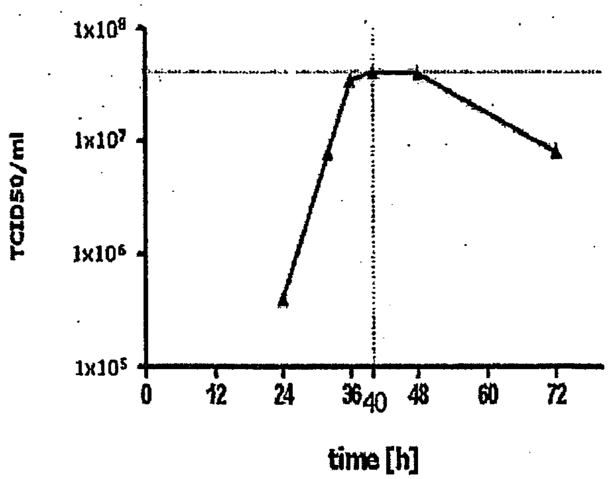


FIGURE 27

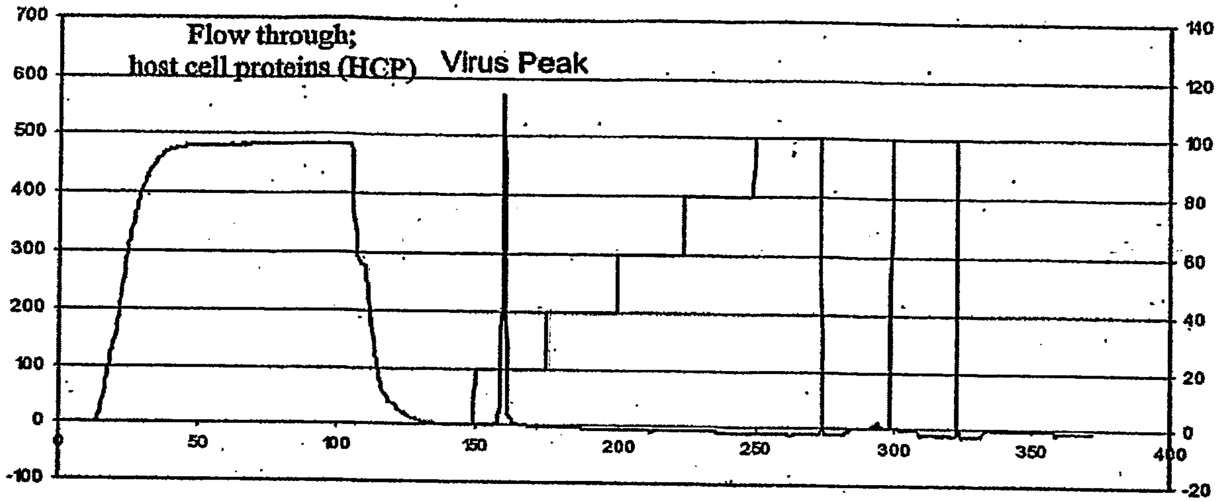


FIGURE 28

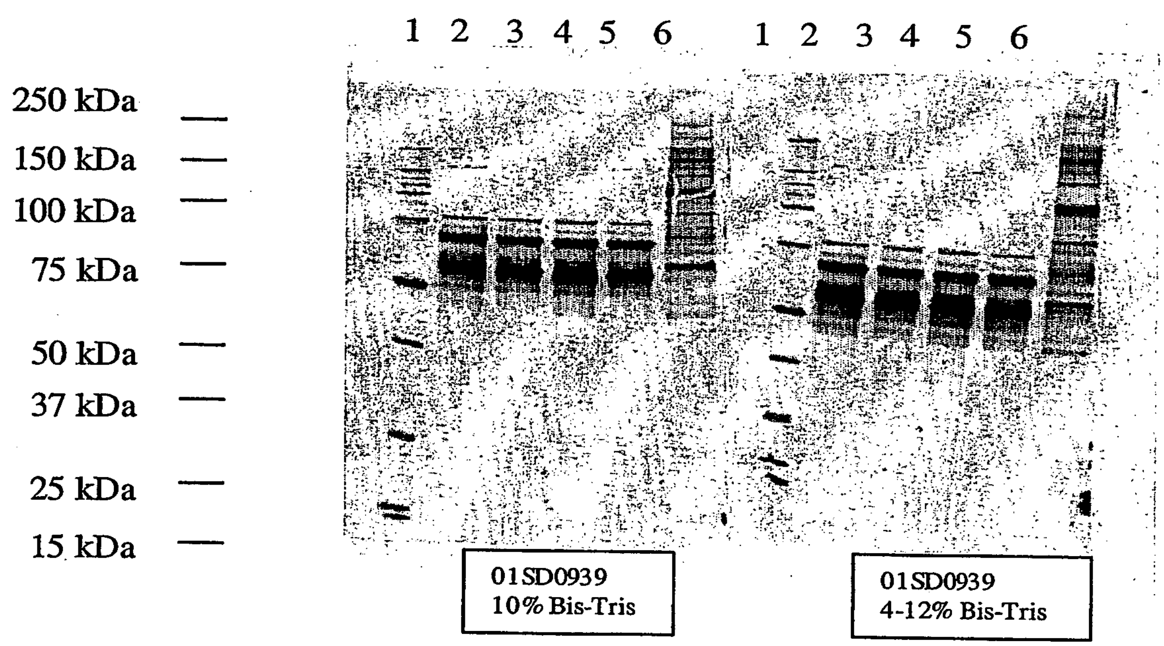


FIGURE 29

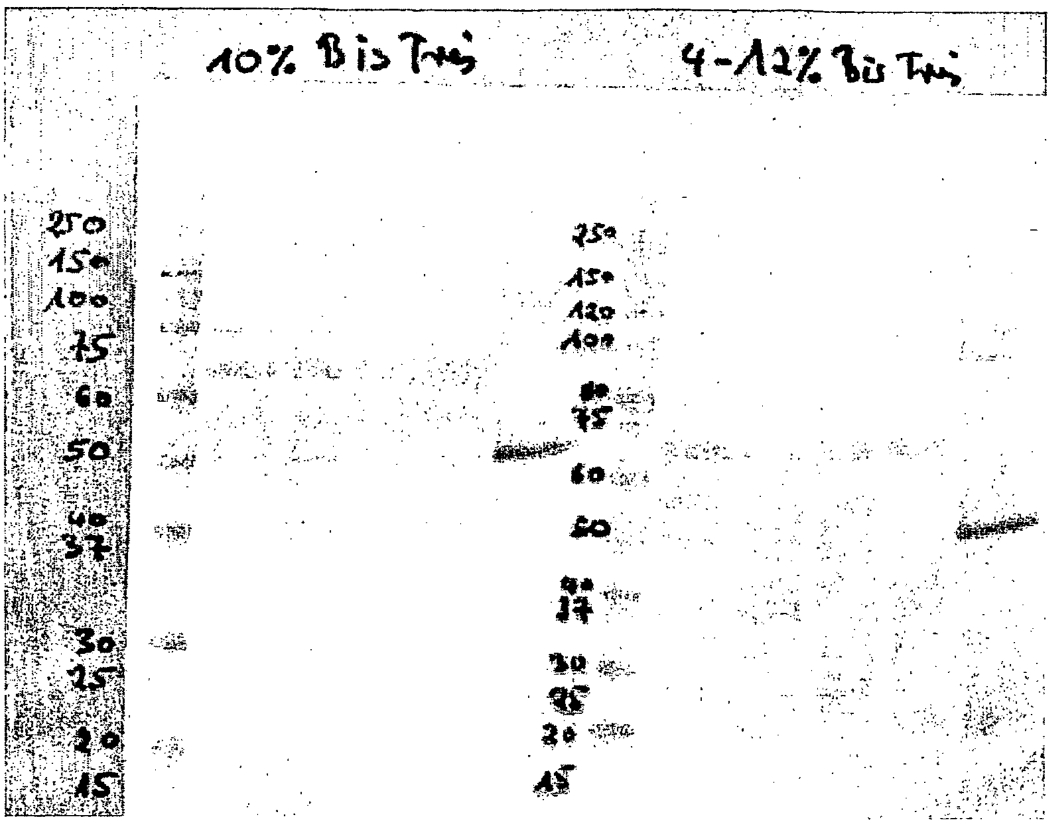


FIGURE 30

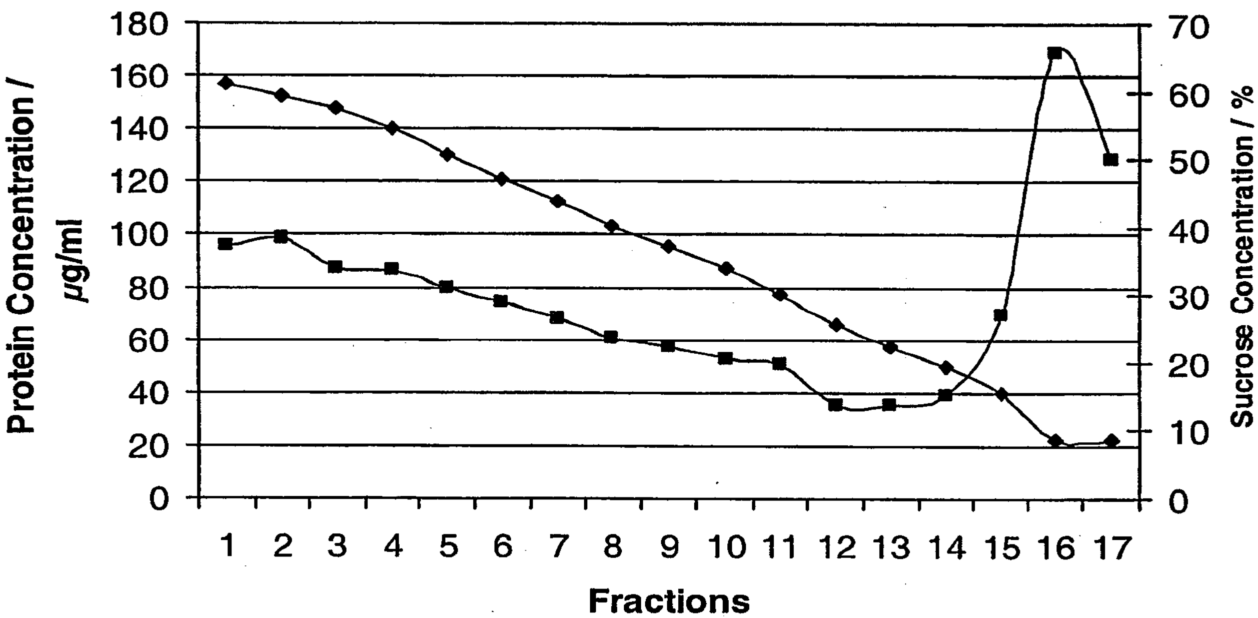


FIGURE 31

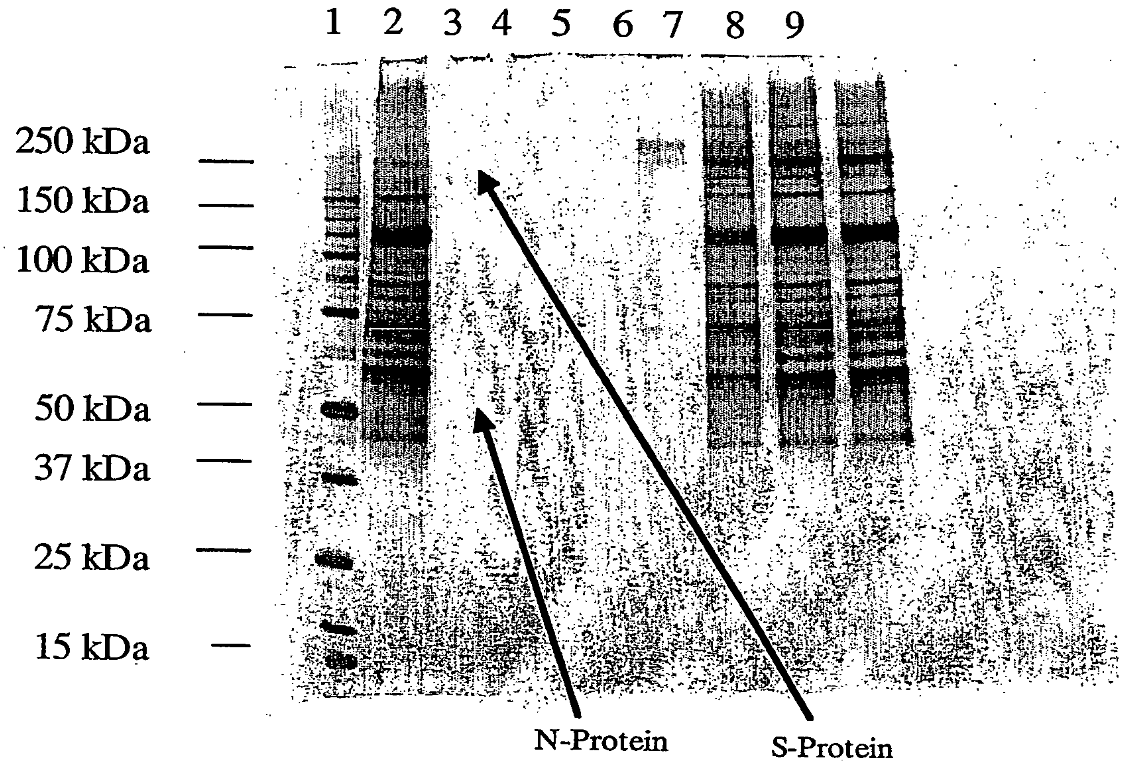


FIGURE 32

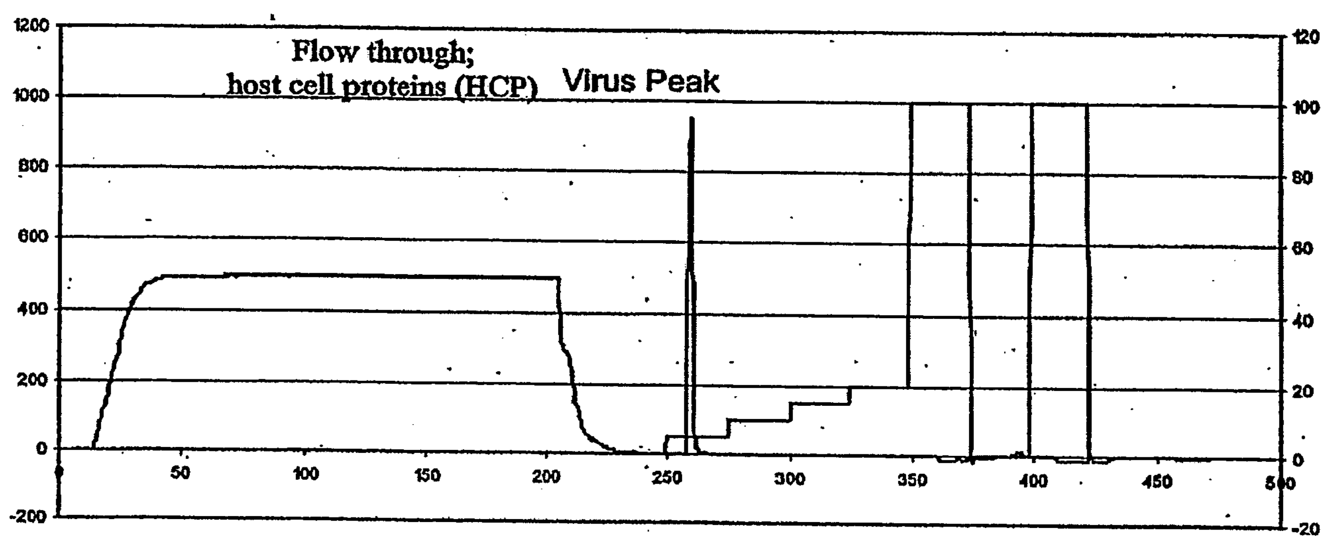


FIGURE 33

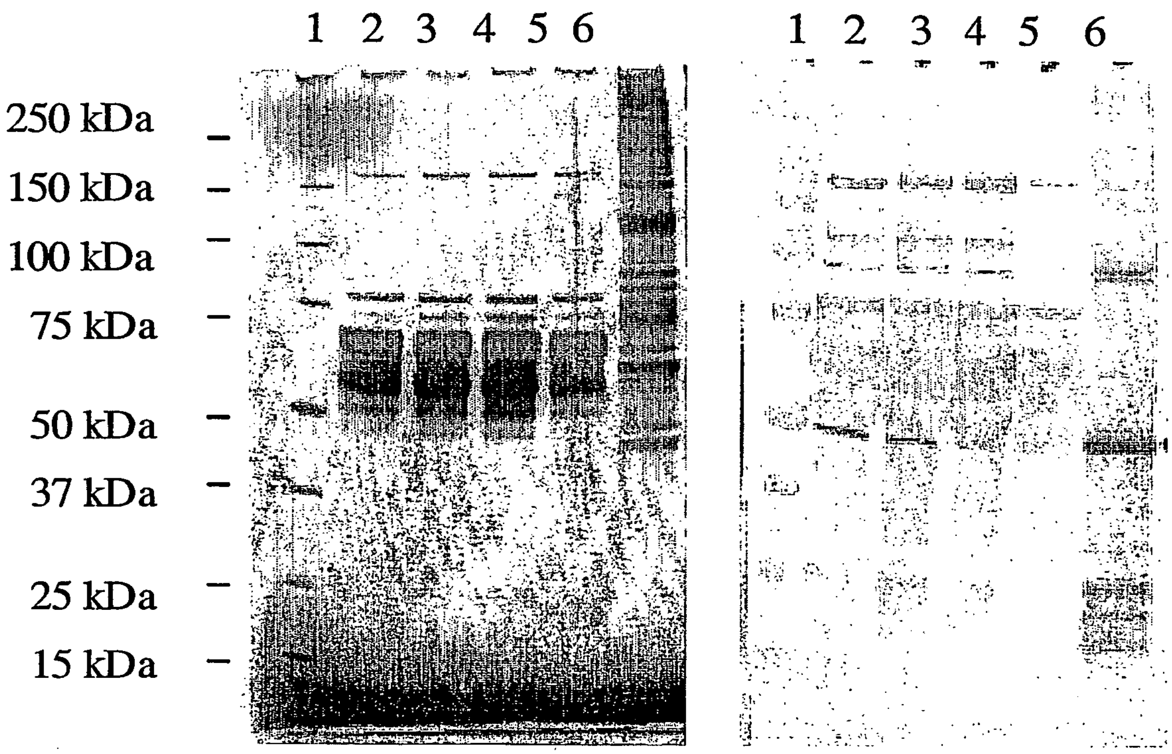


FIGURE 34

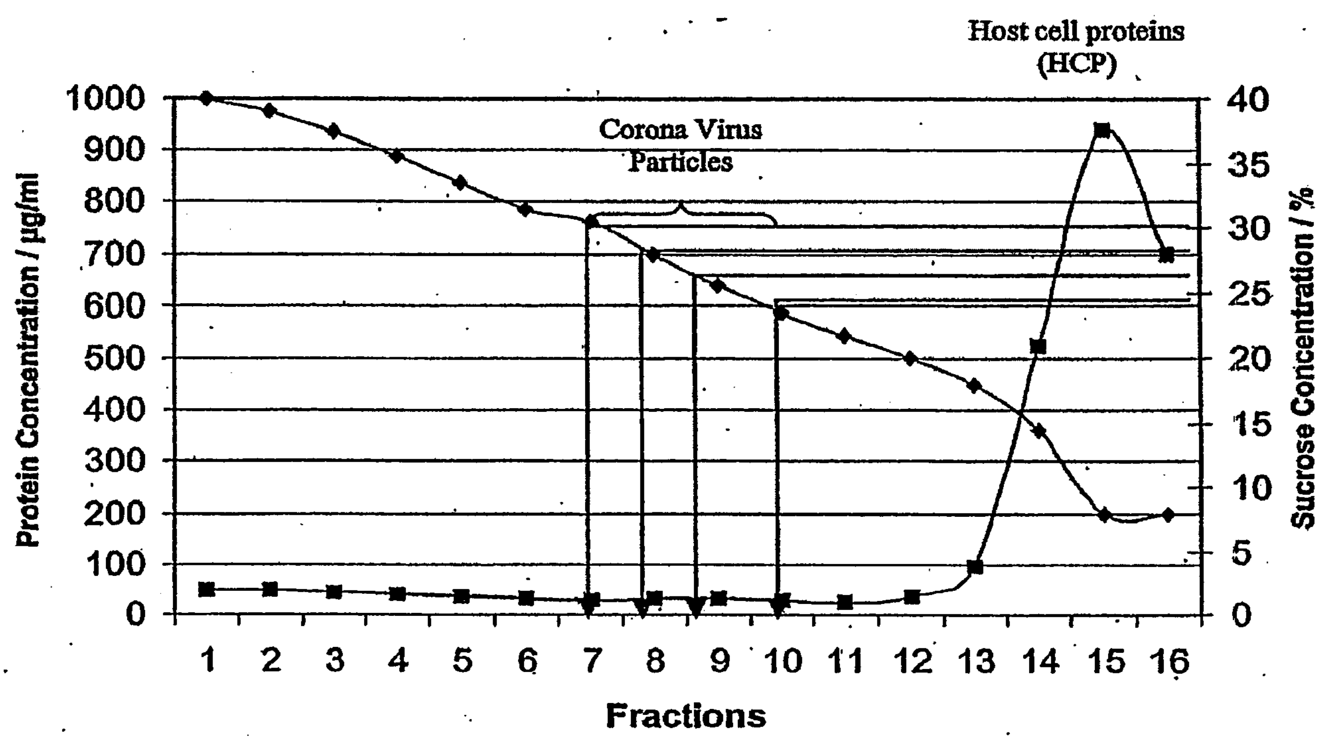


FIGURE 35

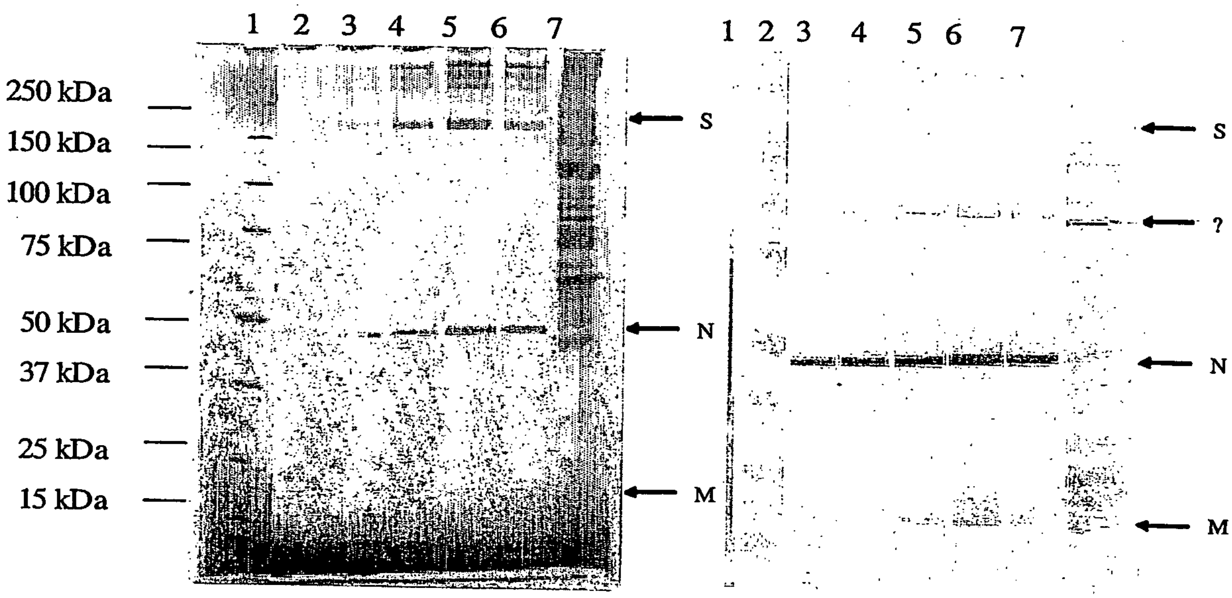


FIGURE 36

FIGURE 36A

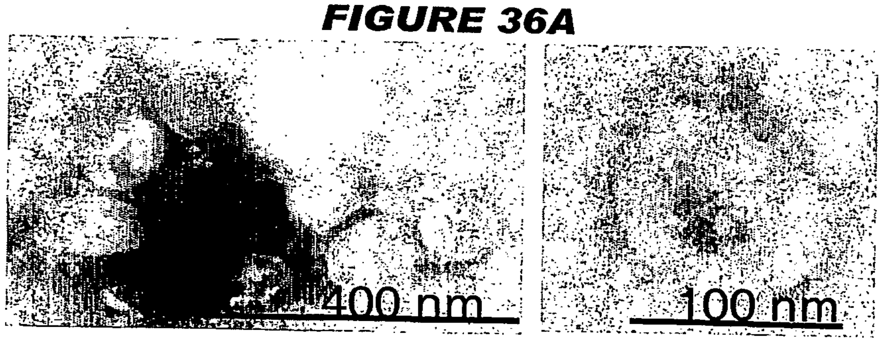


FIGURE 36B

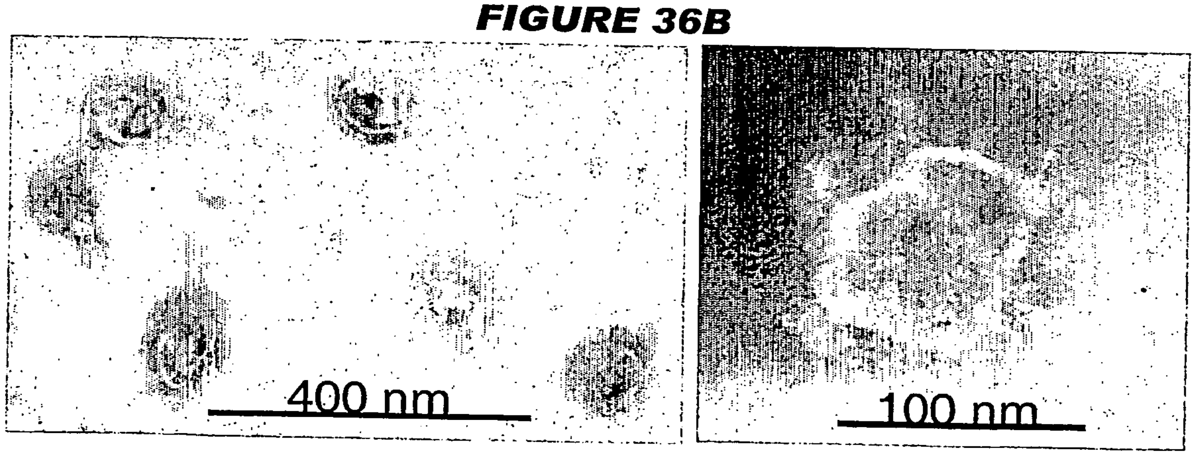


FIGURE 36C

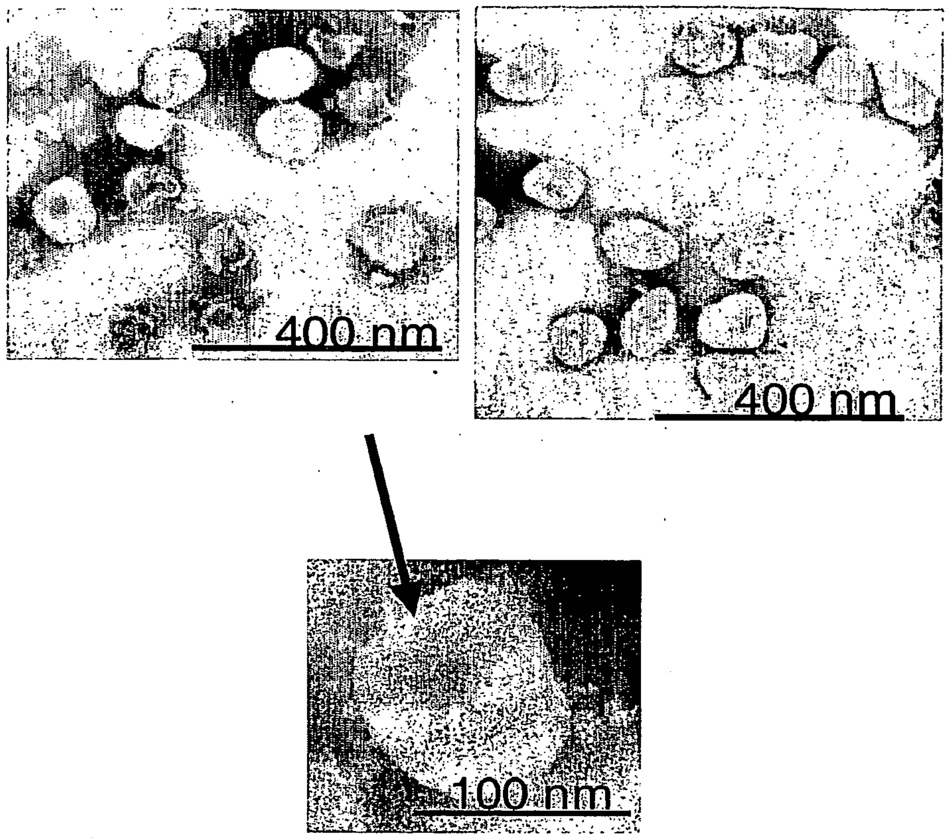


FIGURE 37

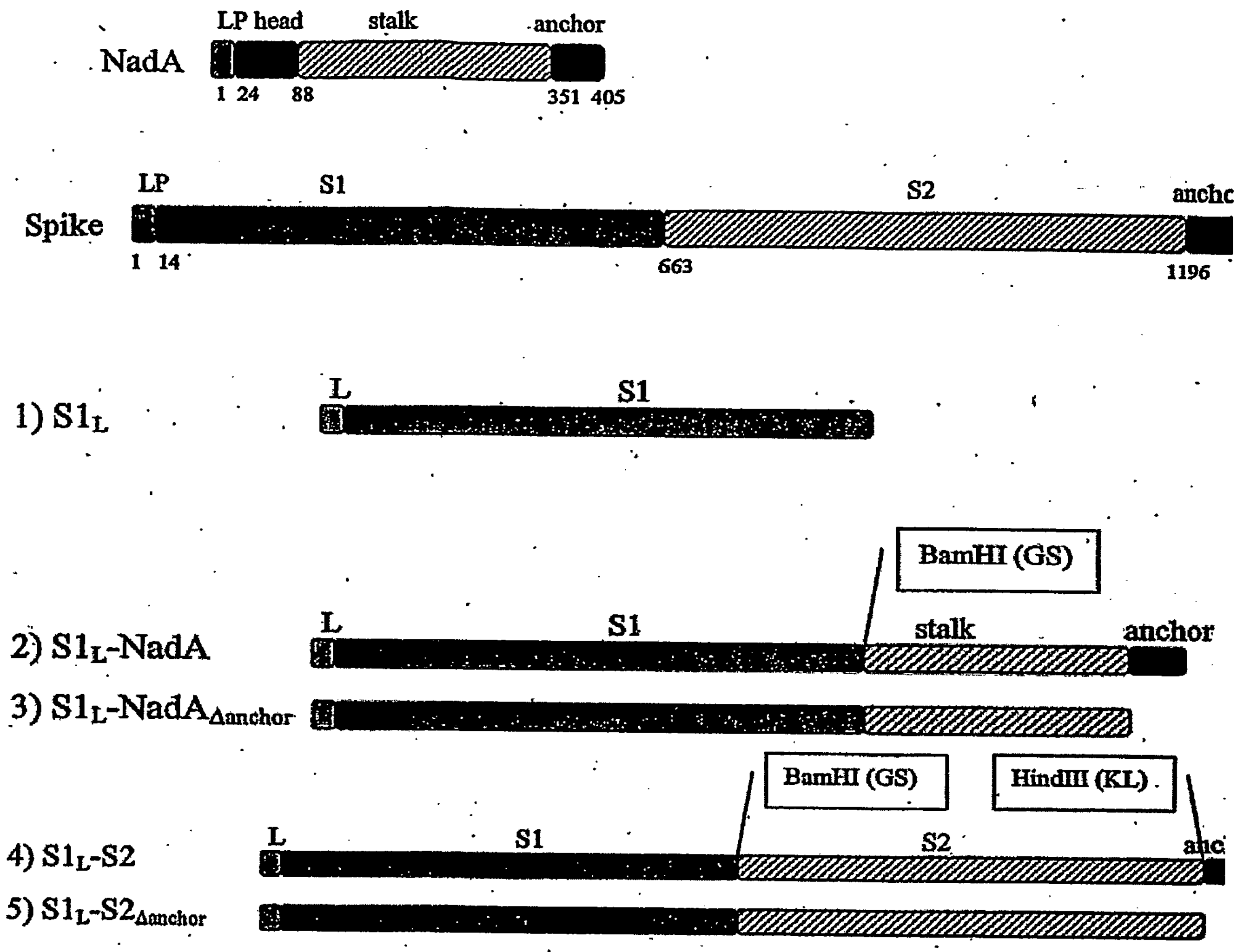

LP head
stalk
anchor
NadA
1 24
88
351 405
LP
S1
S2
anchc
Spike
1 14
663
1196
L
S1
1) S1L
BamHI (GS)
L
S1
stalk
anchor
2) S1L-NadA
3) S1L-NadAΔanchor
BamHI (GS)
HindIII (KL)
L
S1
S2
anc
4) S1L-S2
5) S1L-S2Δanchor

FIGURE 38

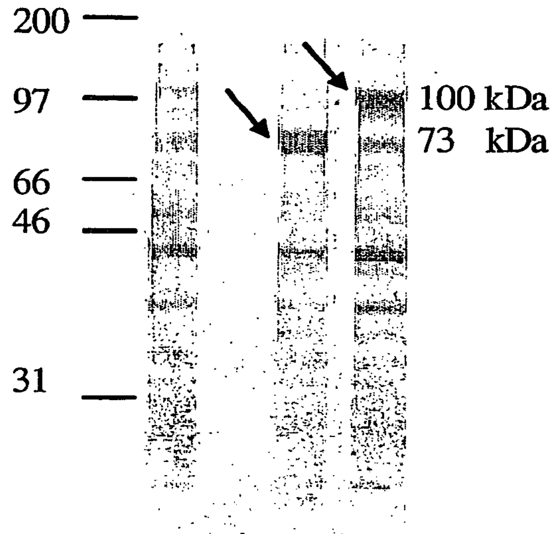


FIGURE 39

FIGURE 39A

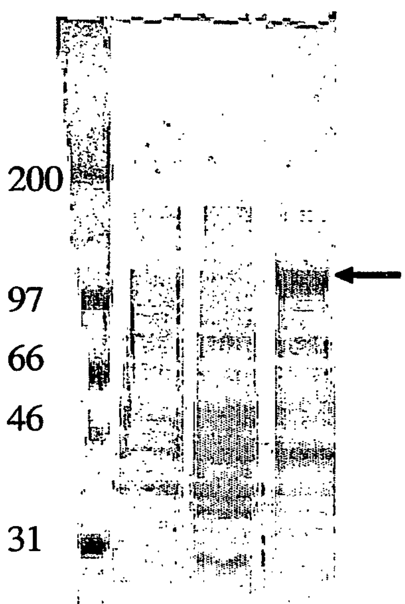


FIGURE 39B

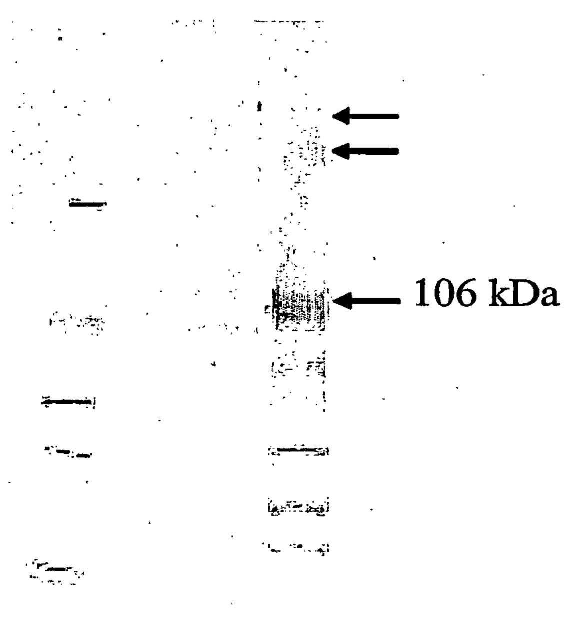


FIGURE 40

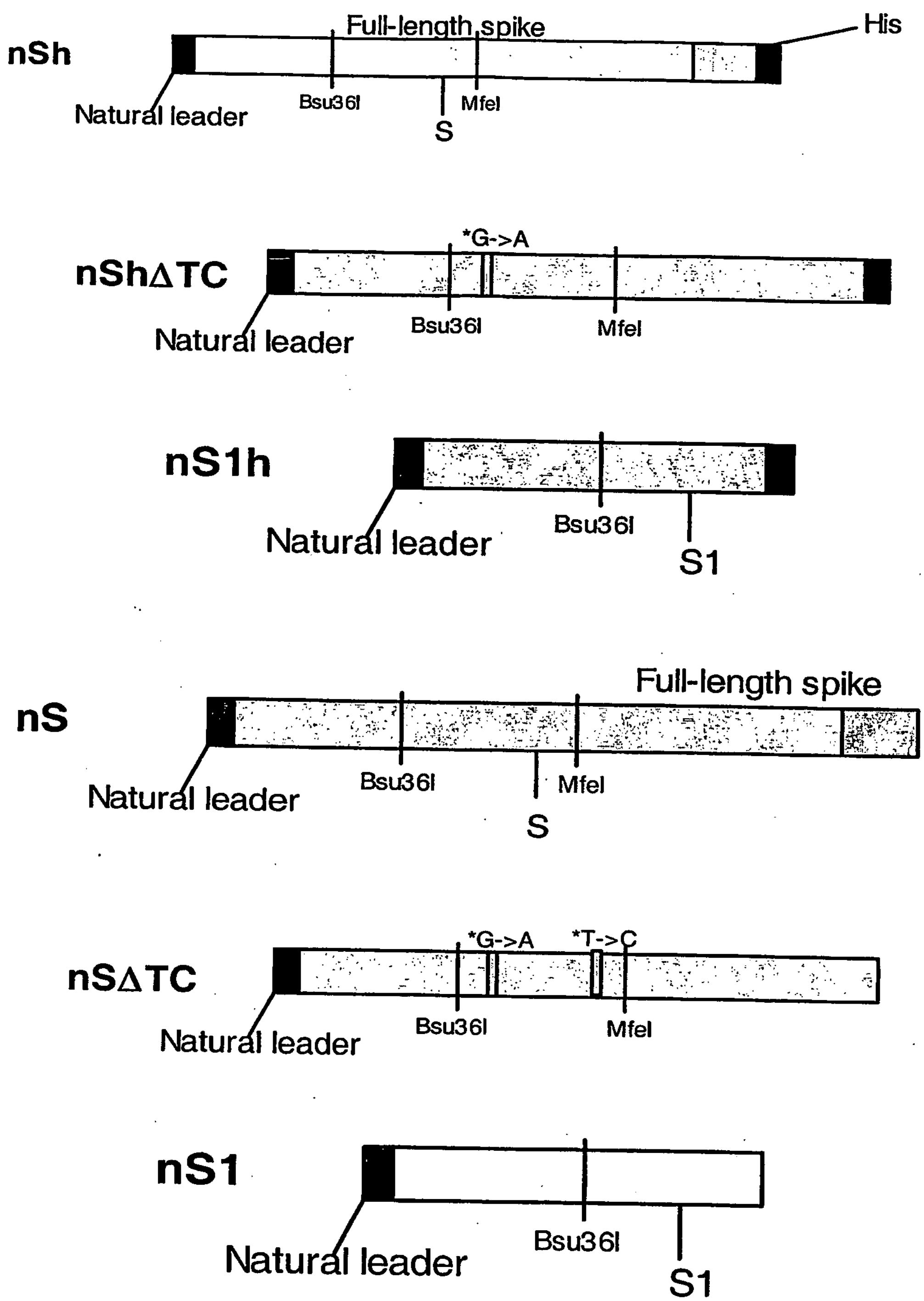

nSh
Full-length spike
His
Natural leader
Bsu36I
Mfel
S
nShΔTC
*G->A
Natural leader
Bsu36I
Mfel
nS1h
Natural leader
Bsu36I
S1
nS
Full-length spike
Natural leader
Bsu36I
Mfel
S
nSΔTC
*G->A
*T->C
Natural leader
Bsu36I
Mfel
nS1
Natural leader
Bsu36I
S1

FIGURE 41

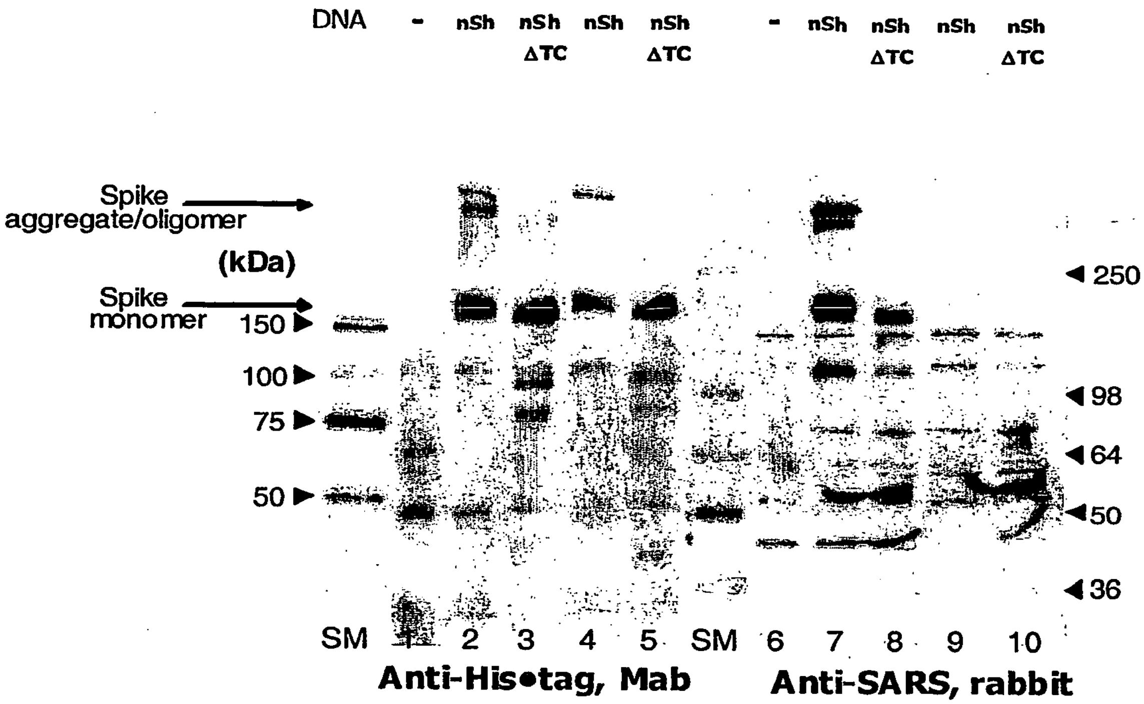


FIGURE 42

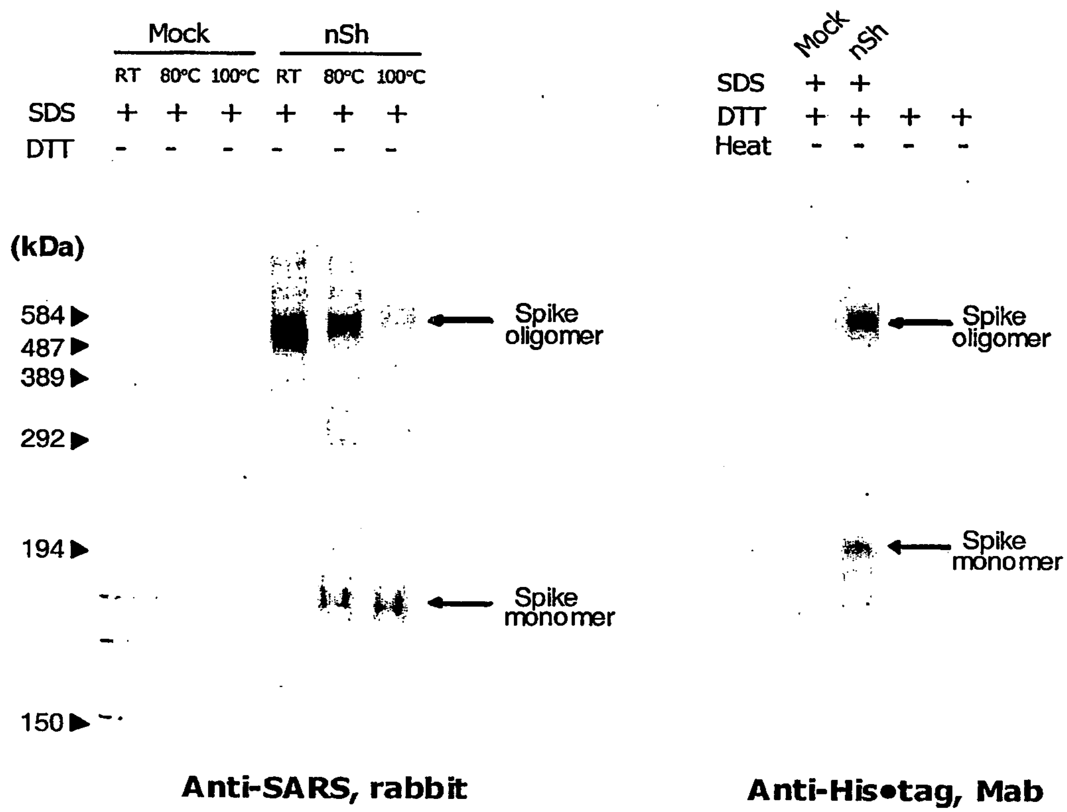


FIGURE 43

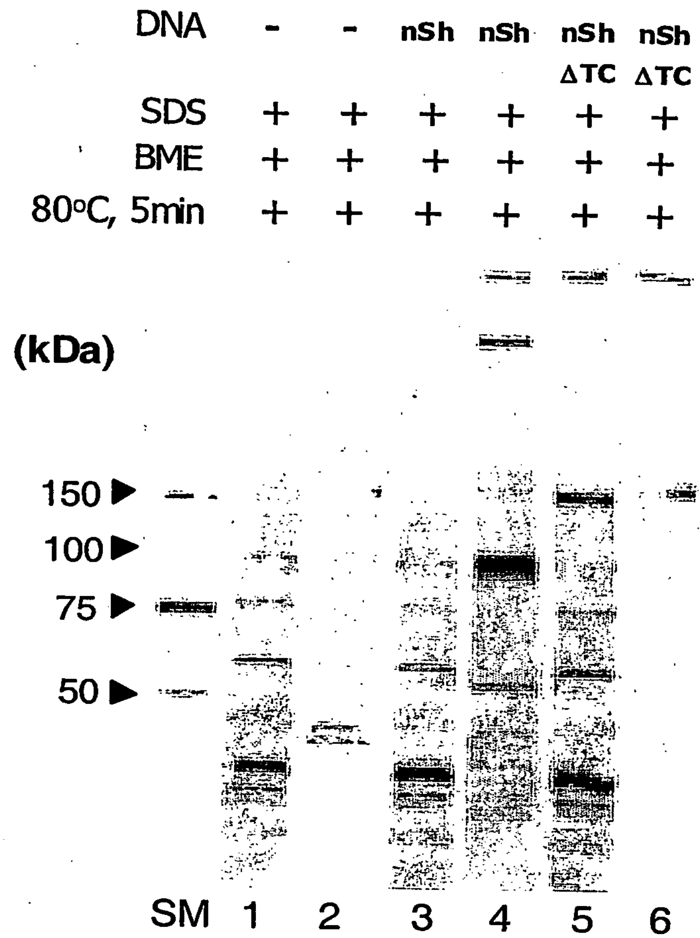

DNA - - nSh nSh nSh ΔTC nSh ΔTC
SDS + + + + + +
BME + + + + + +
80°C, 5min + + + + + +
(kDa)
150
100
75
50
SM 1 2 3 4 5 6

FIGURE 44

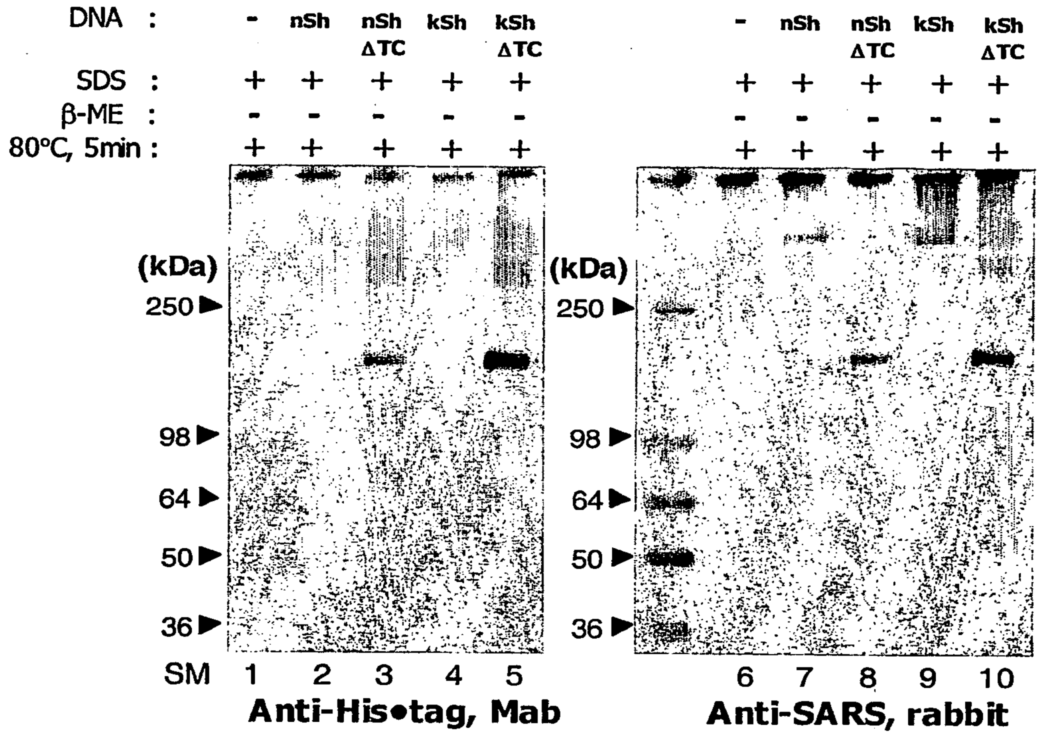


FIGURE 45

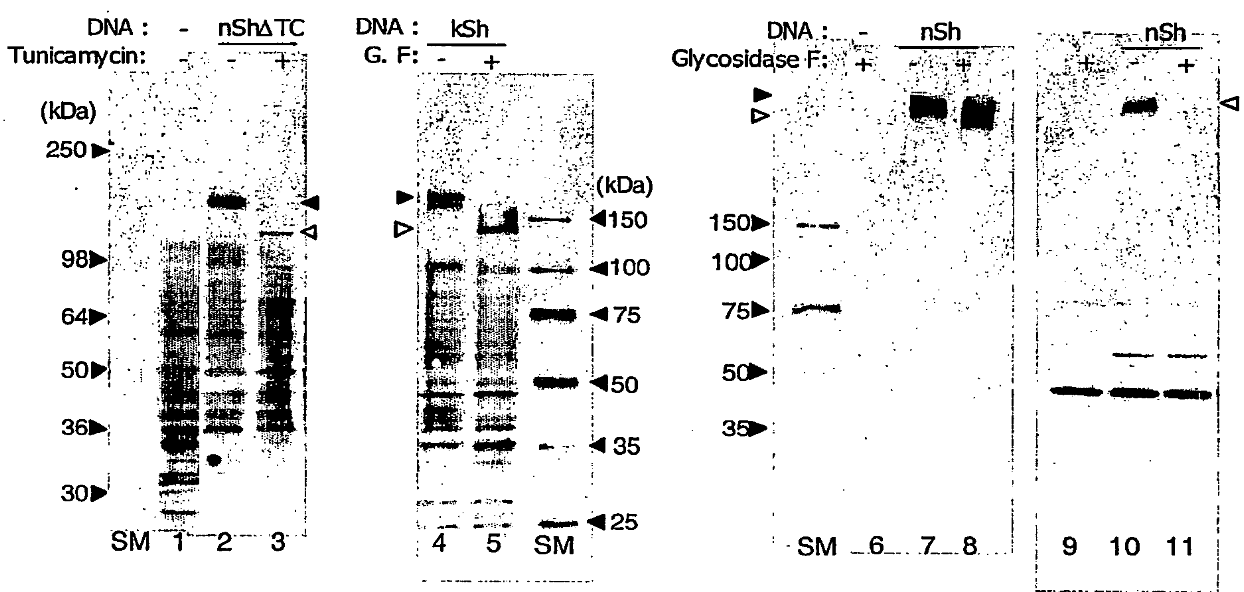


FIGURE 46

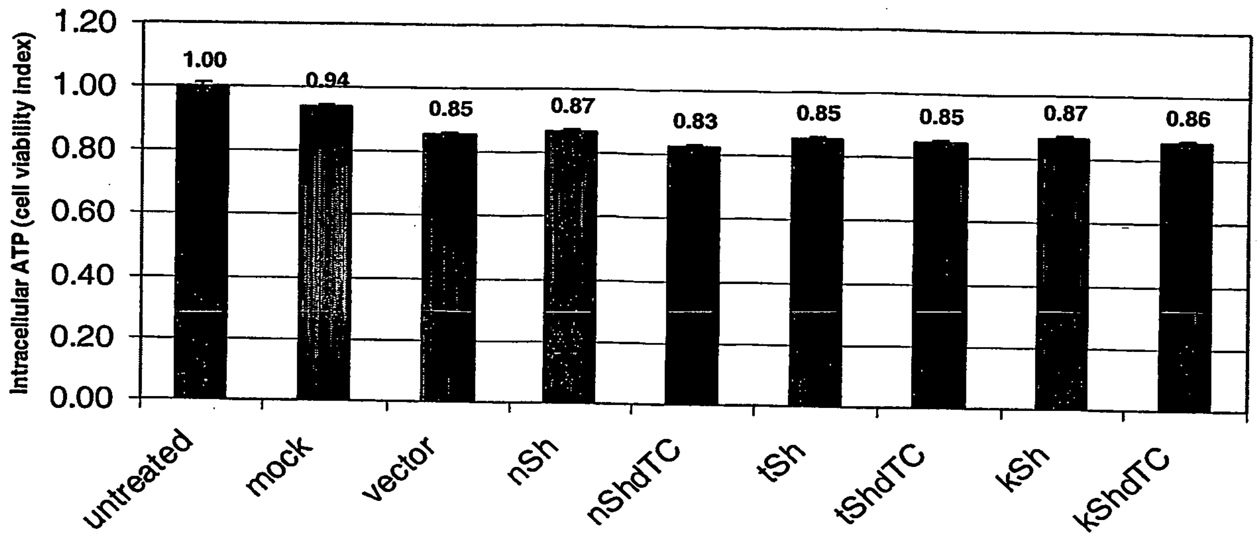


FIGURE 47

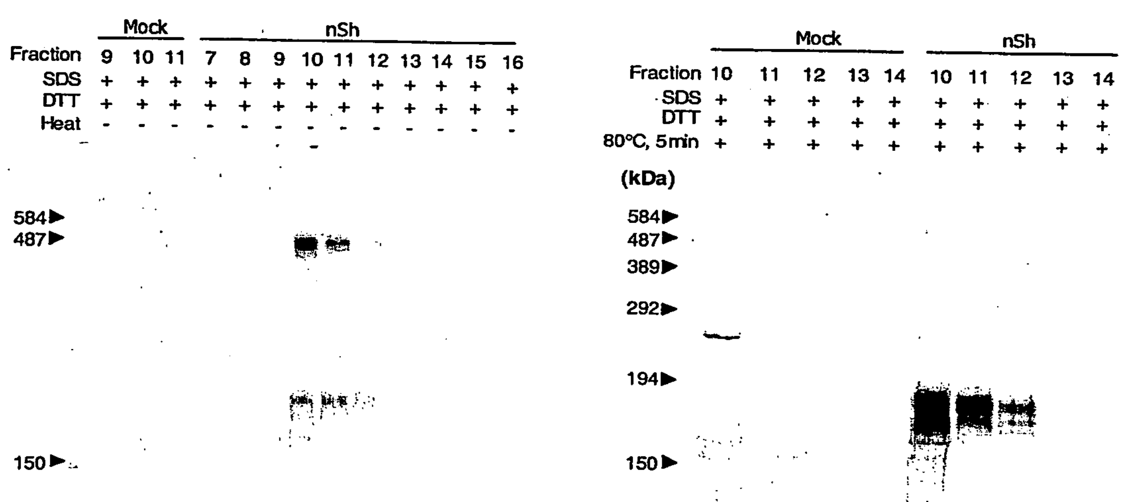


FIGURE 48

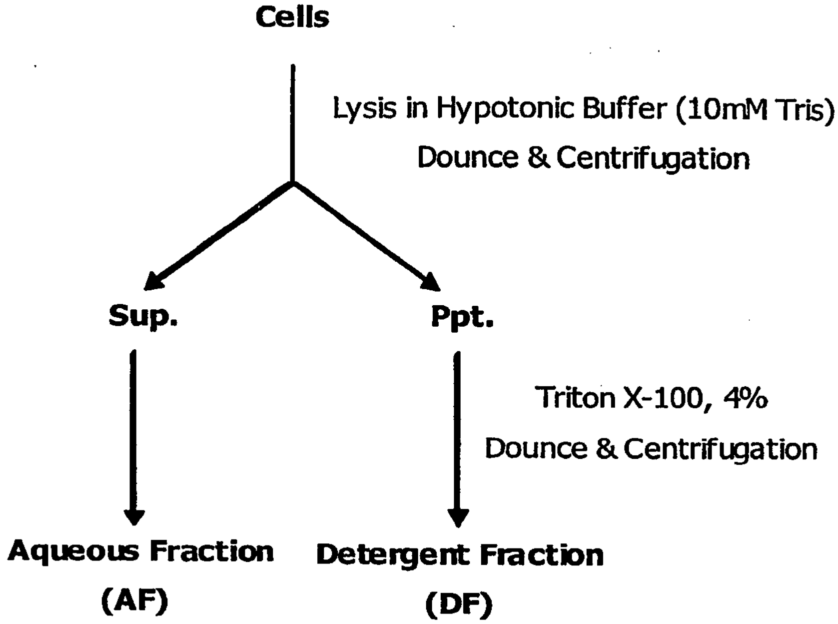


FIGURE 49

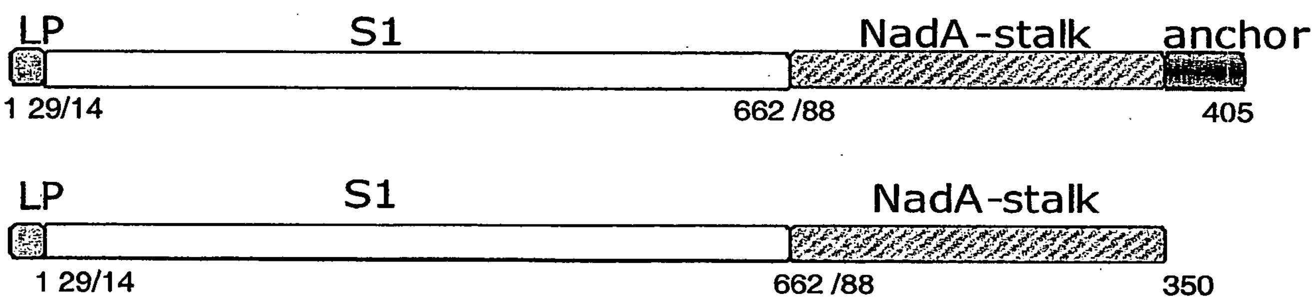
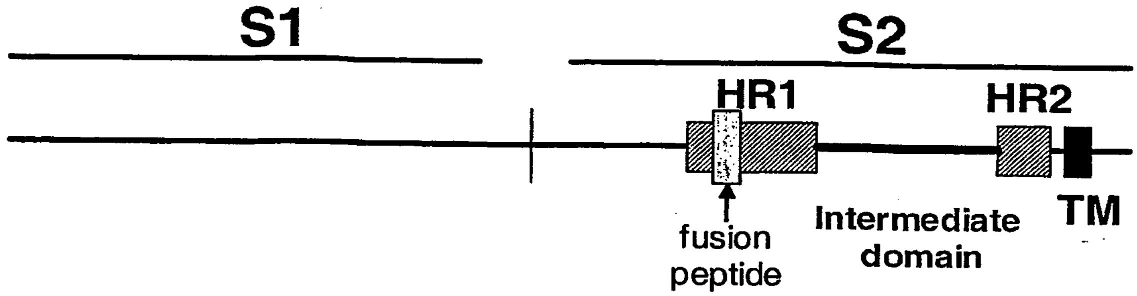


FIGURE 50



a) Leader(NadA)-HR1-GGGGGG-HR2-GGGGSG-stalk(NadA)-anchor(NadA)

b) Leader(NadA)-HR1-GGGGGG-HR2-GGGGSG-stalk(NadA)

c) Leader(NadA)-HR1-intermediate-domain-HR2-GGGGSG-stalk(NadA)-anchor(NadA)

d) Leader(NadA)-HR1-intermediate-domain-HR2- GGGGSG-stalk(NadA)

e) HR1-intermediate-domain-HR2- GGGGSG-stalk(NadA) - HHHHHH

f) Leader(NadA)-HR1-intermediate-domain-HR2-anchor(NadA)

g) Leader(NadA)-HR1-intermediate-domain-HR2

FIGURE 51

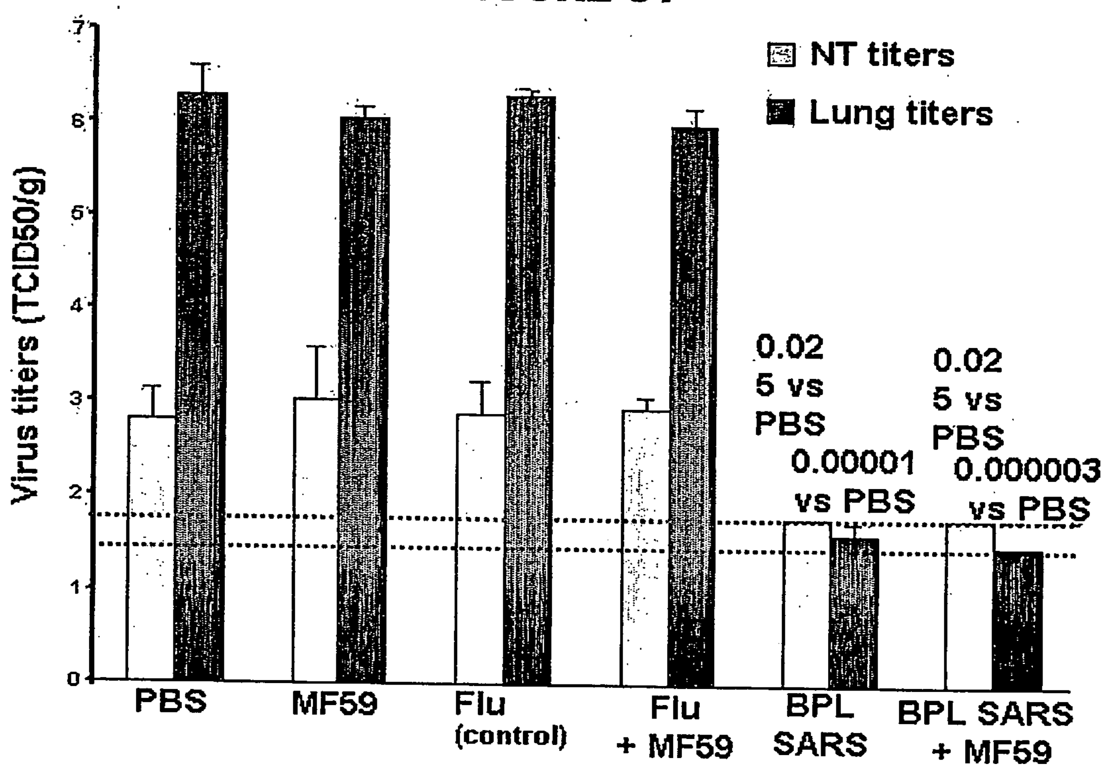


FIGURE 52

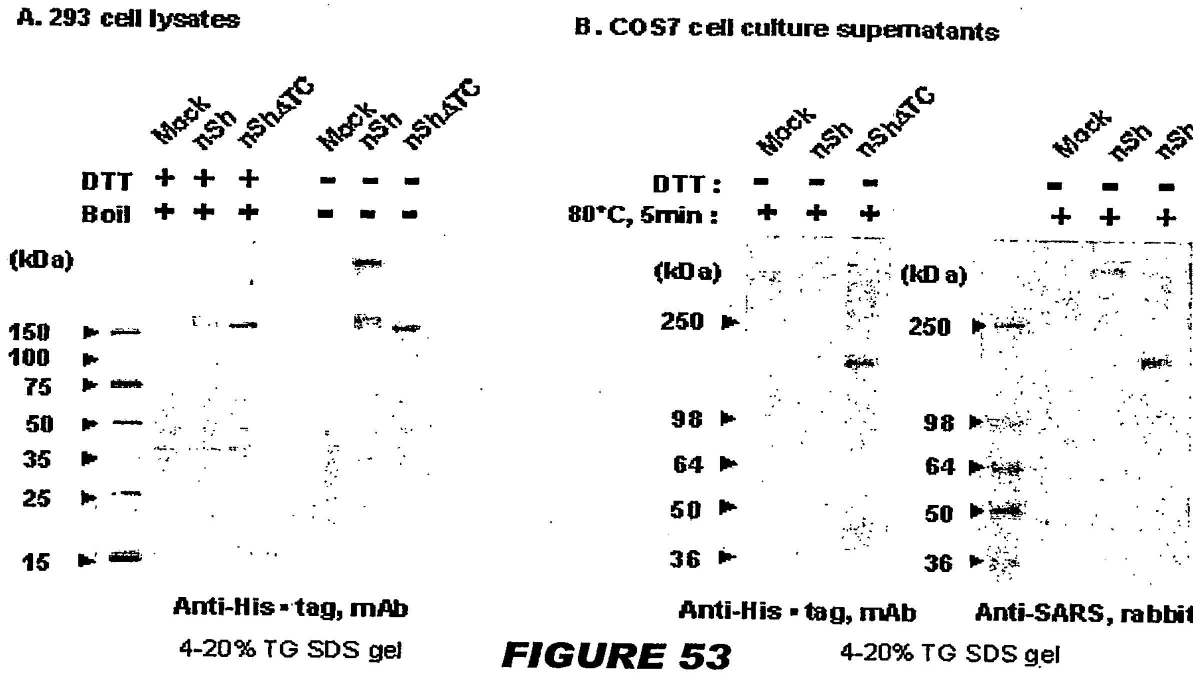


FIGURE 53

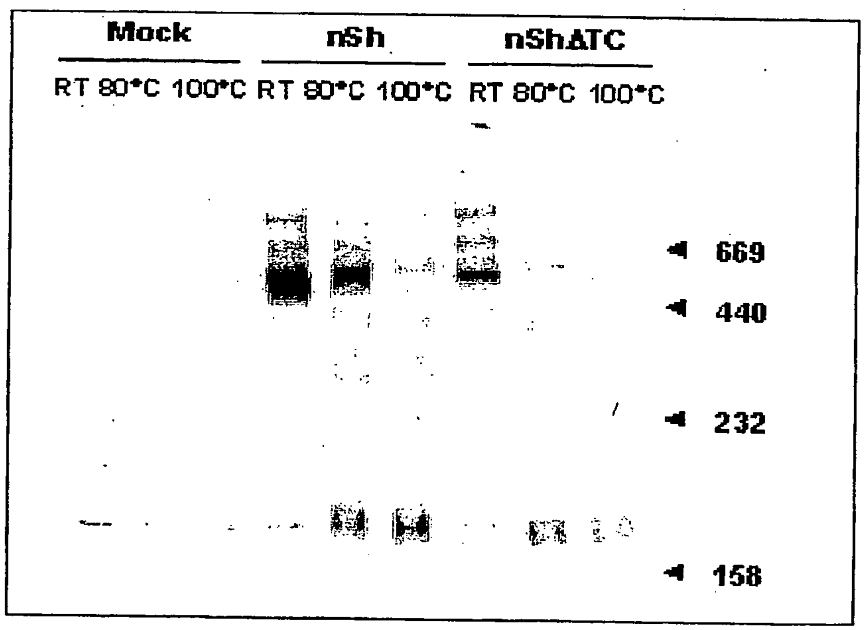


FIGURE 55

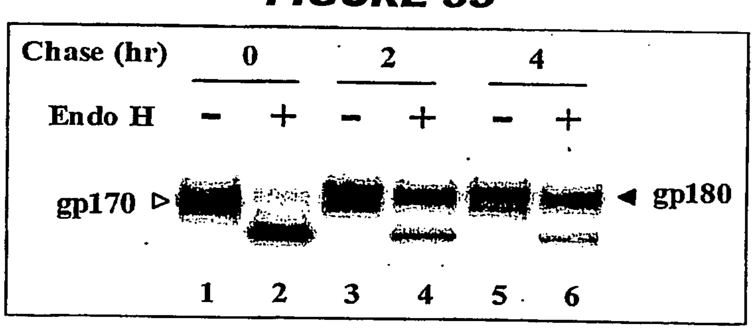


FIGURE 54

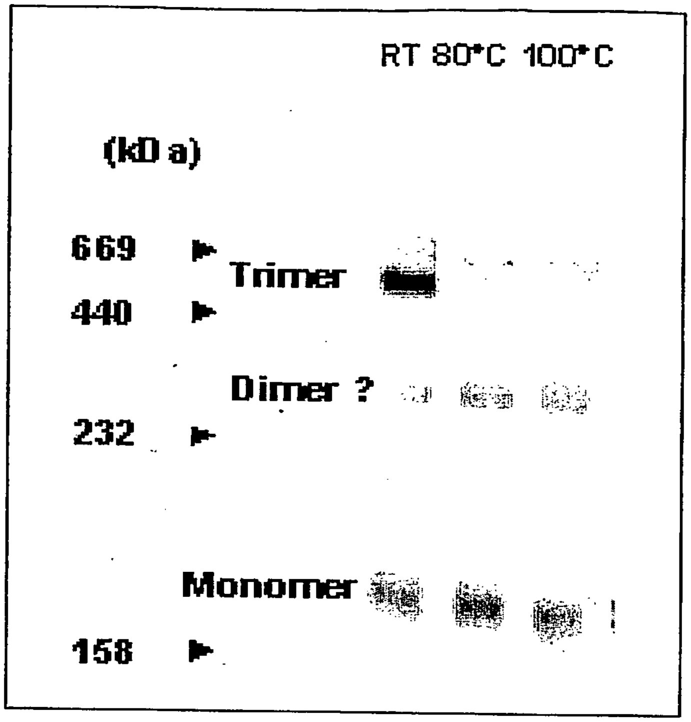


FIGURE 56

FIGURE 56A

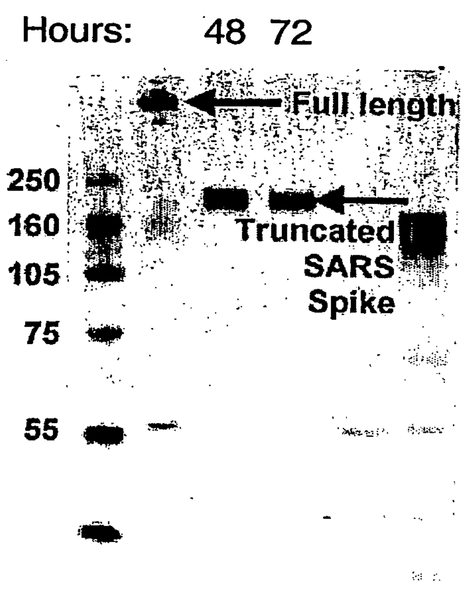


FIGURE 56B

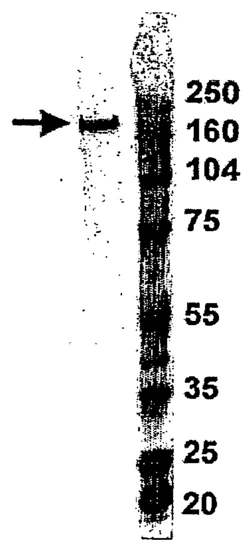


FIGURE 56C

FIGURE 57

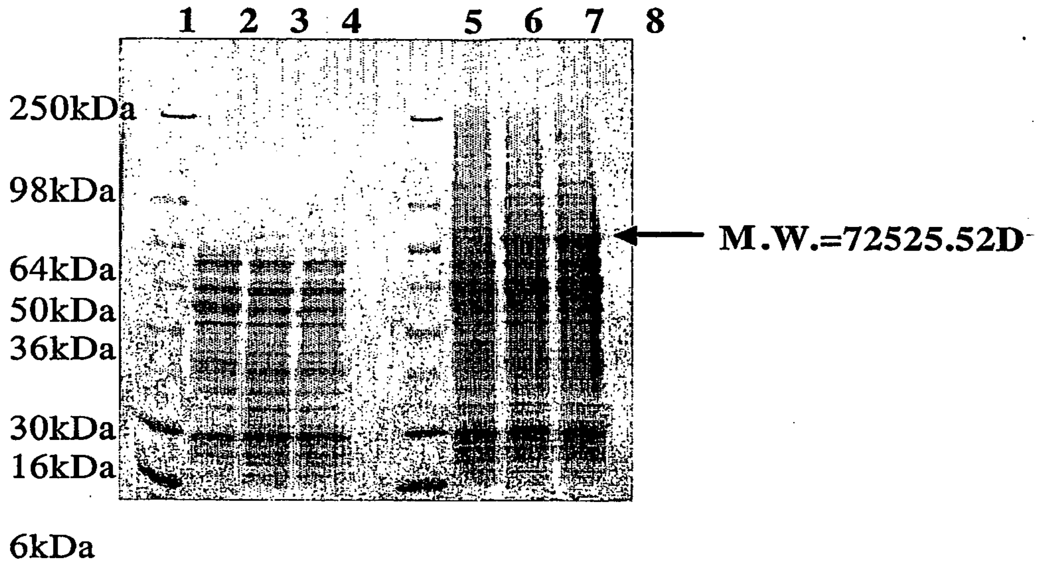


FIGURE 58

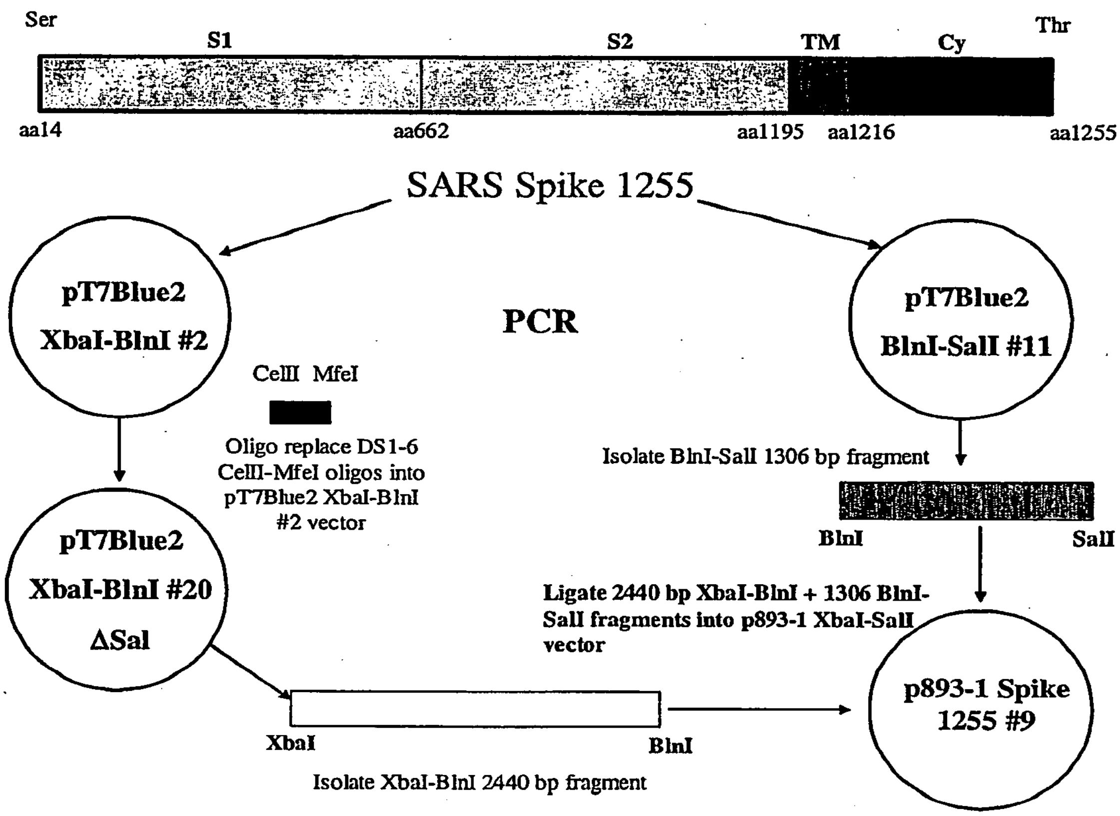


FIGURE 59

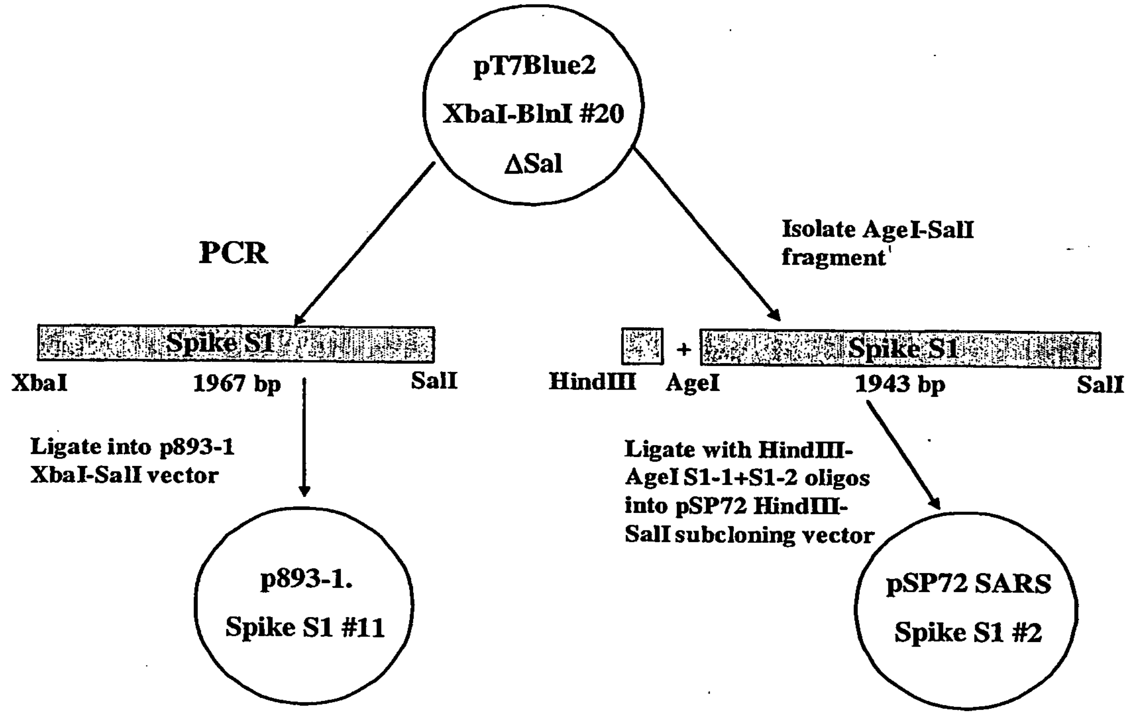


FIGURE 60

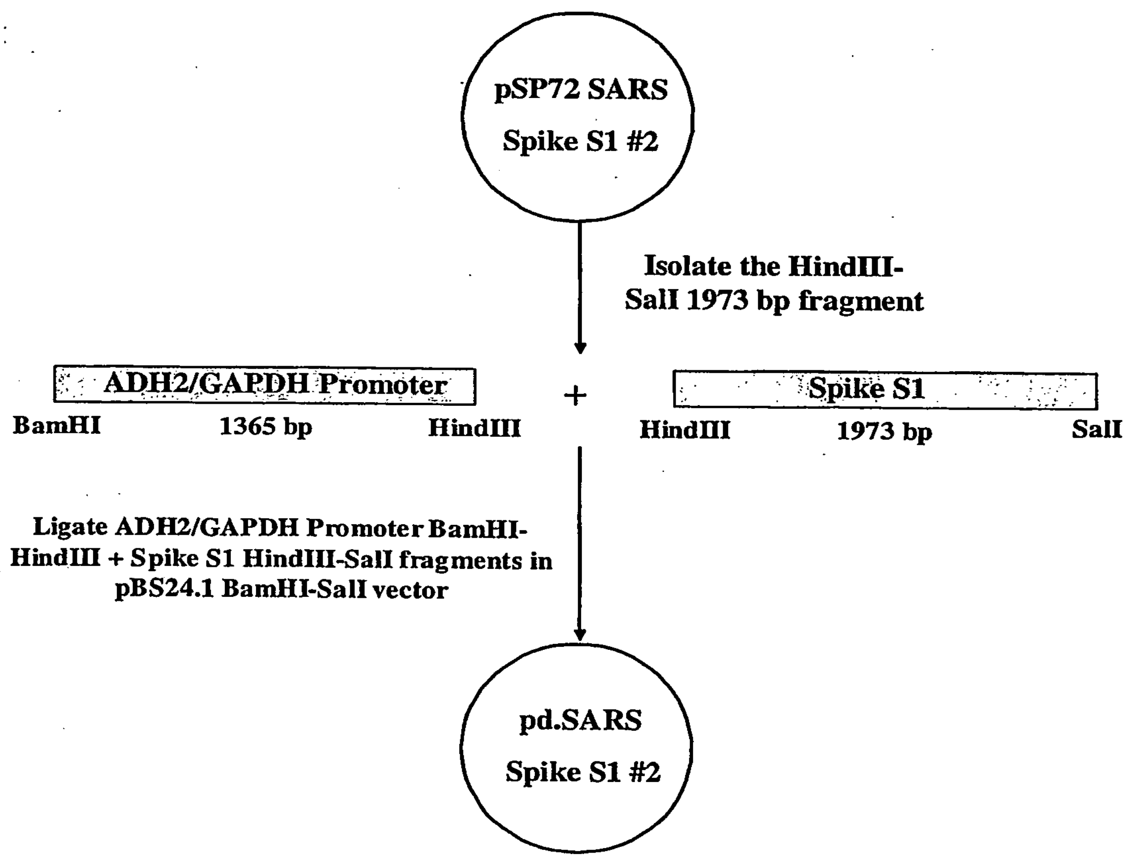


FIGURE 61

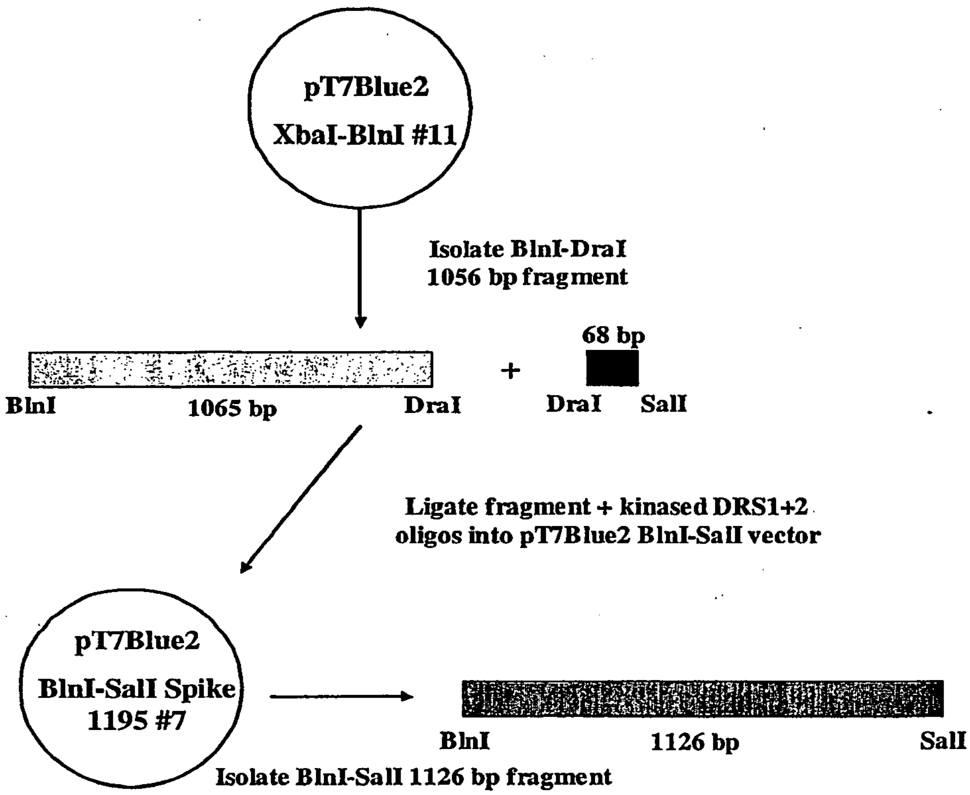


FIGURE 62

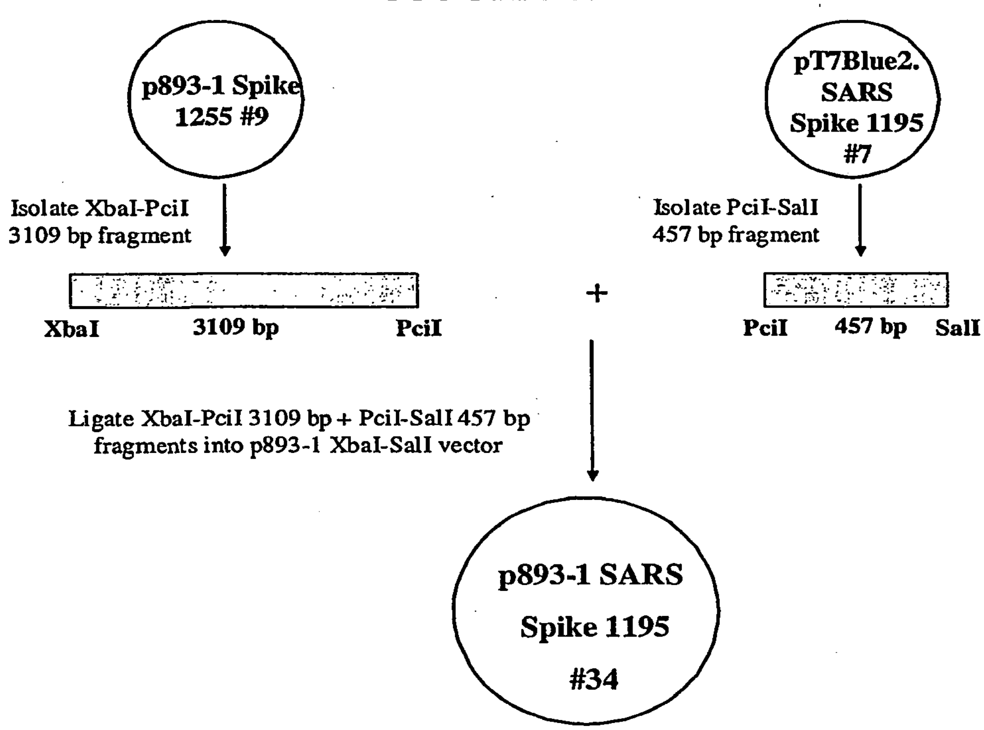


FIGURE 63

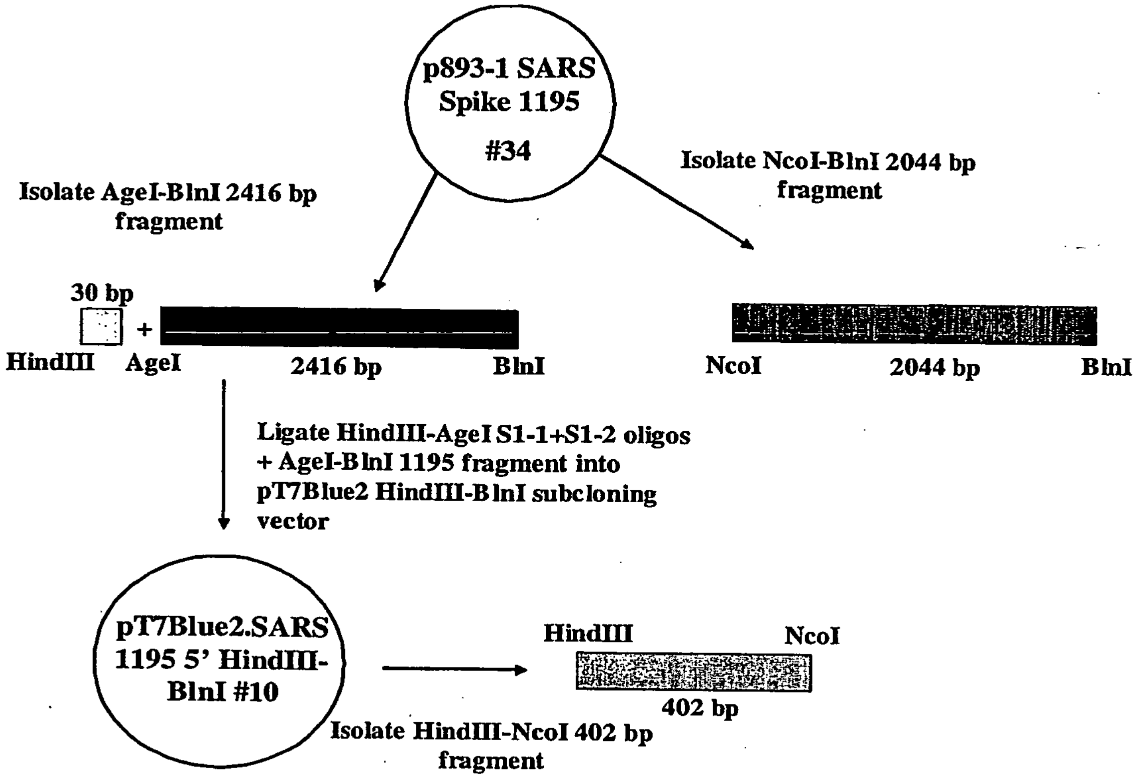


FIGURE 64

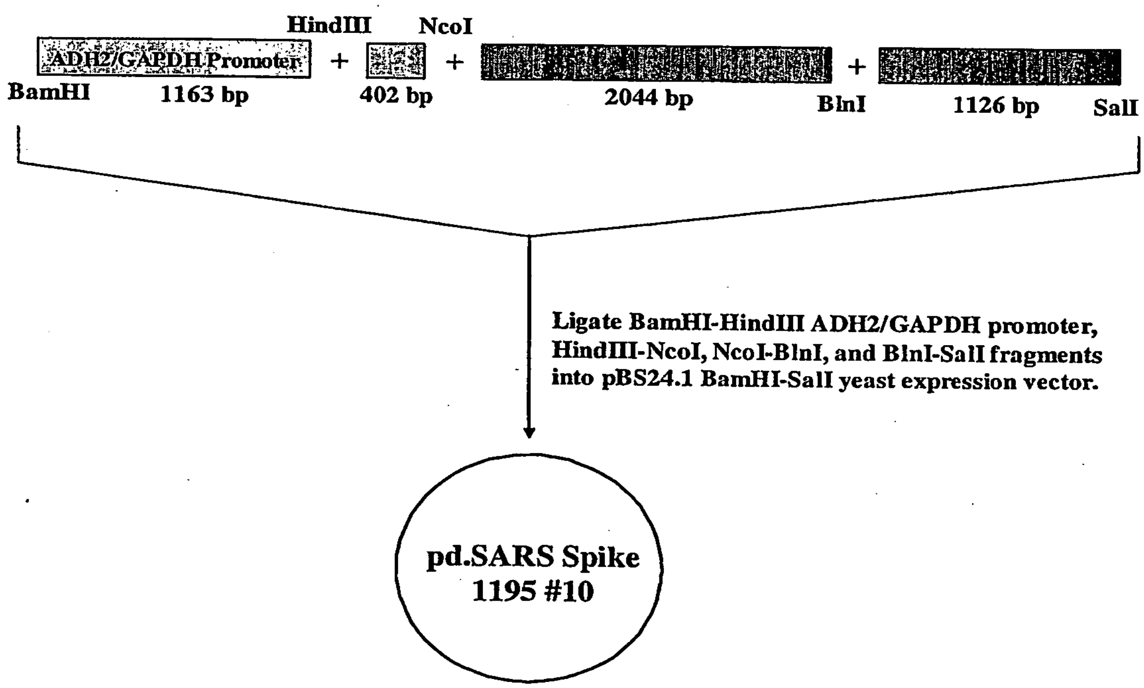


FIGURE 65

```
                   1                                      10
                   M   S   D   L   D   R   C   T   T   F
AAGCTTACAAAACAAA   ATG AGT GAC CTT GAC CGG TGC ACC ACT TTT

                                     20
D   D   V   Q   A   P   N   Y   T   Q   H   T   S   S   M
GAT GAT GTT CAA GCT CCT AAT TAC ACT CAA CAT ACT TCA TCT ATG

                30                                      40
R   G   V   Y   Y   P   D   E   I   F   R   S   D   T   L
AGG GGG GTT TAC TAT CCT GAT GAA ATT TTT AGA TCA GAC ACT CTT

                                     50
Y   L   T   Q   D   L   F   L   P   F   Y   S   N   V   T
TAT TTA ACT CAG GAT TTA TTT CTT CCA TTT TAT TCT AAT GTT ACA

                60                                      70
G   F   H   T   I   N   H   T   F   G   N   P   V   I   P
GGG TTT CAT ACT ATT AAT CAT ACG TTT GGC AAC CCT GTC ATA CCT

                                     80
F   K   D   G   I   Y   F   A   A   T   E   K   S   N   V
TTT AAG GAT GGT ATT TAT TTT GCT GCC ACA GAG AAA TCA AAT GTT

                90                                      100
V   R   G   W   V   F   G   S   T   M   N   N   K   S   Q
GTC CGT GGT TGG GTT TTT GGT TCT ACC ATG AAC AAC AAG TCA CAG

                                     110
S   V   I   I   I   N   N   S   T   N   V   V   I   R   A
TCG GTG ATT ATT ATT AAC AAT TCT ACT AAT GTT GTT ATA CGA GCA

                120                                     130
C   N   F   E   L   C   D   N   P   F   F   A   V   S   K
TGT AAC TTT GAA TTG TGT GAC AAC CCT TTC TTT GCT GTT TCT AAA

                                     140
P   M   G   T   Q   T   H   T   M   I   F   D   N   A   F
CCC ATG GGT ACA CAG ACA CAT ACT ATG ATA TTC GAT AAT GCA TTT

                150                                     160
N   C   T   F   E   Y   I   S   D   A   F   S   L   D   V
AAT TGC ACT TTC GAG TAC ATA TCT GAT GCC TTT TCG CTT GAT GTT

                                     170
S   E   K   S   G   N   F   K   H   L   R   E   F   V   F
TCA GAA AAG TCA GGT AAT TTT AAA CAC TTA CGA GAG TTT GTG TTT

                180                                     190
K   N   K   D   G   F   L   Y   V   Y   K   G   Y   Q   P
AAA AAT AAA GAT GGG TTT CTC TAT GTT TAT AAG GGC TAT CAA CCT
```

```
                                             200
 I   D   V   V   R   D   L   P   S   G   F   N   T   L   K
ATA GAT GTA GTT CGT GAT CTA CCT TCT GGT TTT AAC ACT TTG AAA

                 210                                     220
 P   I   F   K   L   P   L   G   I   N   I   T   N   F   R
CCT ATT TTT AAG TTG CCT CTT GGT ATT AAC ATT ACA AAT TTT AGA

                                     230
 A   I   L   T   A   F   S   P   A   Q   D   I   W   G   T
GCC ATT CTT ACA GCC TTT TCA CCT GCT CAA GAC ATT TGG GGC ACG

                 240                                     250
 S   A   A   A   Y   F   V   G   Y   L   K   P   T   T   F
TCA GCT GCA GCC TAT TTT GTT GGC TAT TTA AAG CCA ACT ACA TTT

                                     260
 M   L   K   Y   D   E   N   G   T   I   T   D   A   V   D
ATG CTC AAG TAT GAT GAA AAT GGT ACA ATC ACA GAT GCT GTT GAT

                 270                                     280
 C   S   Q   N   P   L   A   E   L   K   C   S   V   K   S
TGT TCT CAA AAT CCA CTT GCT GAA CTC AAA TGC TCT GTT AAG AGC

                                     290
 F   E   I   D   K   G   I   Y   Q   T   S   N   F   R   V
TTT GAG ATT GAC AAA GGA ATT TAC CAG ACC TCT AAT TTC AGG GTT

                 300                                     310
 V   P   S   G   D   V   V   R   F   P   N   I   T   N   L
GTT CCC TCA GGA GAT GTT GTG AGA TTC CCT AAT ATT ACA AAC TTG

                                     320
 C   P   F   G   E   V   F   N   A   T   K   F   P   S   V
TGT CCT TTT GGA GAG GTT TTT AAT GCT ACT AAA TTC CCT TCT GTC

                 330                                     340
 Y   A   W   E   R   K   K   I   S   N   C   V   A   D   Y
TAT GCA TGG GAG AGA AAA AAA ATT TCT AAT TGT GTT GCT GAT TAC

                                     350
 S   V   L   Y   N   S   T   F   F   S   T   F   K   C   Y
TCT GTG CTC TAC AAC TCA ACA TTT TTT TCA ACC TTT AAG TGC TAT

                 360                                     370
 G   V   S   A   T   K   L   N   D   L   C   F   S   N   V
GGC GTT TCT GCC ACT AAG TTG AAT GAT CTT TGC TTC TCC AAT GTC

                                     380
 Y   A   D   S   F   V   V   K   G   D   D   V   R   Q   I
TAT GCA GAT TCT TTT GTA GTC AAG GGA GAT GAT GTA AGA CAA ATA

                 390                                     400
 A   P   G   Q   T   G   V   I   A   D   Y   N   Y   K   L
GCG CCA GGG CAA ACT GGT GTT ATT GCT GAT TAT AAT TAT AAA TTG
```

410
P D D F M G C V L A W N T R N
CCA GAT GAT TTC ATG GGT TGT GTC CTT GCT TGG AAT ACT AGG AAC

420 430
I D A T S T G N Y N Y K Y R Y
ATT GAT GCT ACT TCA ACT GGT AAT TAT AAT TAT AAA TAT AGG TAT

440
L R H G K L R P F E R D I S N
CTT AGA CAT GGC AAG CTT AGG CCC TTT GAG AGA GAC ATA TCT AAT

450 460
V P F S P D G K P C T P P A L
GTG CCT TTC TCC CCT GAT GGC AAA CCT TGC ACC CCA CCT GCT CTT

470
N C Y W P L N D Y G F Y T T T
AAT TGT TAT TGG CCA TTA AAT GAT TAT GGT TTT TAC ACC ACT ACT

480 490
G I G Y Q P Y R V V V L S F E
GGC ATT GGC TAC CAA CCT TAC AGA GTT GTA GTA CTT TCT TTT GAA

500
L L N A P A T V C G P K L S T
CTT TTA AAT GCA CCG GCC ACG GTT TGT GGA CCA AAA TTA TCC ACT

510 520
D L I K N Q C V N F N F N G L
GAC CTT ATT AAG AAC CAG TGT GTC AAT TTT AAT TTT AAT GGA CTC

530
T G T G V L T P S S K R F Q P
ACT GGT ACT GGT GTG TTA ACT CCT TCT TCA AAG AGA TTT CAA CCA

540 550
F Q Q F G R D V S D F T D S V
TTT CAA CAA TTT GGC CGT GAT GTT TCT GAT TTC ACT GAT TCC GTT

560
R D P K T S E I L D I S P C S
CGA GAT CCT AAA ACA TCT GAA ATA TTA GAC ATT TCA CCT TGC TCT

570 580
F G G V S V I T P G T N A S S
TTT GGG GGT GTA AGT GTA ATT ACA CCT GGA ACA AAT GCT TCA TCT

590
E V A V L Y Q D V N C T D V S
GAA GTT GCT GTT CTA TAT CAA GAT GTT AAC TGC ACT GAT GTT TCT

600 610
T A I H A D Q L T P A W R I Y
ACA GCA ATT CAT GCA GAT CAA CTC ACA CCA GCT TGG CGC ATA TAT

```
                                          620
 S   T   G   N   N   V   F   Q   T   Q   A   G   C   L   I
TCT ACT GGA AAC AAT GTA TTC CAG ACT CAA GCA GGC TGT CTT ATA

                630                                      640
 G   A   E   H   V   D   T   S   Y   E   C   D   I   P   I
GGA GCT GAG CAT GTT GAT ACT TCT TAT GAG TGC GAC ATT CCT ATT

                                     650
 G   A   G   I   C   A   S   Y   H   T   OC            SEQ ID NO: 9799
GGA GCT GGC ATT TGT GCT AGT TAC CAT ACA TAA TGAGTCGAC SEQ ID NO: 9800

Translated Mol. Weight = 72525.52
```

FIGURE 66

```
                           1                                      10
                           M   S   D   L   D   R   C   T   T   F
AAGCTTACAAAACAAA          ATG AGT GAC CTT GAC CGG TGC ACC ACT TTT

                                         20
 D   D   V   Q   A   P   N   Y   T   Q   H   T   S   S   M
GAT GAT GTT CAA GCT CCT AAT TAC ACT CAA CAT ACT TCA TCT ATG

                30                                       40
 R   G   V   Y   Y   P   D   E   I   F   R   S   D   T   L
AGG GGG GTT TAC TAT CCT GAT GAA ATT TTT AGA TCA GAC ACT CTT

                                         50
 Y   L   T   Q   D   L   F   L   P   F   Y   S   N   V   T
TAT TTA ACT CAG GAT TTA TTT CTT CCA TTT TAT TCT AAT GTT ACA

                60                                       70
 G   F   H   T   I   N   H   T   F   G   N   P   V   I   P
GGG TTT CAT ACT ATT AAT CAT ACG TTT GGC AAC CCT GTC ATA CCT

                                         80
 F   K   D   G   I   Y   F   A   A   T   E   K   S   N   V
TTT AAG GAT GGT ATT TAT TTT GCT GCC ACA GAG AAA TCA AAT GTT

                90                                      100
 V   R   G   W   V   F   G   S   T   M   N   N   K   S   Q
GTC CGT GGT TGG GTT TTT GGT TCT ACC ATG AAC AAC AAG TCA CAG

                                        110
 S   V   I   I   I   N   N   S   T   N   V   V   I   R   A
TCG GTG ATT ATT ATT AAC AAT TCT ACT AAT GTT GTT ATA CGA GCA

               120                                      130
 C   N   F   E   L   C   D   N   P   F   F   A   V   S   K
TGT AAC TTT GAA TTG TGT GAC AAC CCT TTC TTT GCT GTT TCT AAA

                                        140
 P   M   G   T   Q   T   H   T   M   I   F   D   N   A   F
CCC ATG GGT ACA CAG ACA CAT ACT ATG ATA TTC GAT AAT GCA TTT

               150                                      160
 N   C   T   F   E   Y   I   S   D   A   F   S   L   D   V
AAT TGC ACT TTC GAG TAC ATA TCT GAT GCC TTT TCG CTT GAT GTT
```

```
                                    170
 S   E   K   S   G   N   F   K   H   L   R   E   F   V   F
TCA GAA AAG TCA GGT AAT TTT AAA CAC TTA CGA GAG TTT GTG TTT

                180                                     190
 K   N   K   D   G   F   L   Y   V   Y   K   G   Y   Q   P
AAA AAT AAA GAT GGG TTT CTC TAT GTT TAT AAG GGC TAT CAA CCT

                                    200
 I   D   V   V   R   D   L   P   S   G   F   N   T   L   K
ATA GAT GTA GTT CGT GAT CTA CCT TCT GGT TTT AAC ACT TTG AAA

                210                                     220
 P   I   F   K   L   P   L   G   I   N   I   T   N   F   R
CCT ATT TTT AAG TTG CCT CTT GGT ATT AAC ATT ACA AAT TTT AGA

                                    230
 A   I   L   T   A   F   S   P   A   Q   D   I   W   G   T
GCC ATT CTT ACA GCC TTT TCA CCT GCT CAA GAC ATT TGG GGC ACG

                240                                     250
 S   A   A   A   Y   F   V   G   Y   L   K   P   T   T   F
TCA GCT GCA GCC TAT TTT GTT GGC TAT TTA AAG CCA ACT ACA TTT

                                    260
 M   L   K   Y   D   E   N   G   T   I   T   D   A   V   D
ATG CTC AAG TAT GAT GAA AAT GGT ACA ATC ACA GAT GCT GTT GAT

                270                                     280
 C   S   Q   N   P   L   A   E   L   K   C   S   V   K   S
TGT TCT CAA AAT CCA CTT GCT GAA CTC AAA TGC TCT GTT AAG AGC

                                    290
 F   E   I   D   K   G   I   Y   Q   T   S   N   F   R   V
TTT GAG ATT GAC AAA GGA ATT TAC CAG ACC TCT AAT TTC AGG GTT

                300                                     310
 V   P   S   G   D   V   V   R   F   P   N   I   T   N   L
GTT CCC TCA GGA GAT GTT GTG AGA TTC CCT AAT ATT ACA AAC TTG

                                    320
 C   P   F   G   E   V   F   N   A   T   K   F   P   S   V
TGT CCT TTT GGA GAG GTT TTT AAT GCT ACT AAA TTC CCT TCT GTC

                330                                     340
 Y   A   W   E   R   K   K   I   S   N   C   V   A   D   Y
TAT GCA TGG GAG AGA AAA AAA ATT TCT AAT TGT GTT GCT GAT TAC

                                    350
 S   V   L   Y   N   S   T   F   F   S   T   F   K   C   Y
TCT GTG CTC TAC AAC TCA ACA TTT TTT TCA ACC TTT AAG TGC TAT

                360                                     370
 G   V   S   A   T   K   L   N   D   L   C   F   S   N   V
GGC GTT TCT GCC ACT AAG TTG AAT GAT CTT TGC TTC TCC AAT GTC

                                    380
 Y   A   D   S   F   V   V   K   G   D   D   V   R   Q   I
TAT GCA GAT TCT TTT GTA GTC AAG GGA GAT GAT GTA AGA CAA ATA

                390                                     400
 A   P   G   Q   T   G   V   I   A   D   Y   N   Y   K   L
GCG CCA GGG CAA ACT GGT GTT ATT GCT GAT TAT AAT TAT AAA TTG
```

410
P D D F M G C V L A W N T R N
CCA GAT GAT TTC ATG GGT TGT GTC CTT GCT TGG AAT ACT AGG AAC

420 430
I D A T S T G N Y N Y K Y R Y
ATT GAT GCT ACT TCA ACT GGT AAT TAT AAT TAT AAA TAT AGG TAT

440
L R H G K L R P F E R D I S N
CTT AGA CAT GGC AAG CTT AGG CCC TTT GAG AGA GAC ATA TCT AAT

450 460
V P F S P D G K P C T P P A L
GTG CCT TTC TCC CCT GAT GGC AAA CCT TGC ACC CCA CCT GCT CTT

470
N C Y W P L N D Y G F Y T T T
AAT TGT TAT TGG CCA TTA AAT GAT TAT GGT TTT TAC ACC ACT ACT

480 490
G I G Y Q P Y R V V V L S F E
GGC ATT GGC TAC CAA CCT TAC AGA GTT GTA GTA CTT TCT TTT GAA

500
L L N A P A T V C G P K L S T
CTT TTA AAT GCA CCG GCC ACG GTT TGT GGA CCA AAA TTA TCC ACT

510 520
D L I K N Q C V N F N F N G L
GAC CTT ATT AAG AAC CAG TGT GTC AAT TTT AAT TTT AAT GGA CTC

530
T G T G V L T P S S K R F Q P
ACT GGT ACT GGT GTG TTA ACT CCT TCT TCA AAG AGA TTT CAA CCA

540 550
F Q Q F G R D V S D F T D S V
TTT CAA CAA TTT GGC CGT GAT GTT TCT GAT TTC ACT GAT TCC GTT

560
R D P K T S E I L D I S P C S
CGA GAT CCT AAA ACA TCT GAA ATA TTA GAC ATT TCA CCT TGC TCT

570 580
F G G V S V I T P G T N A S S
TTT GGG GGT GTA AGT GTA ATT ACA CCT GGA ACA AAT GCT TCA TCT

590
E V A V L Y Q D V N C T D V S
GAA GTT GCT GTT CTA TAT CAA GAT GTT AAC TGC ACT GAT GTT TCT

600 610
T A I H A D Q L T P A W R I Y
ACA GCA ATT CAT GCA GAT CAA CTC ACA CCA GCT TGG CGC ATA TAT

620
S T G N N V F Q T Q A G C L I
TCT ACT GGA AAC AAT GTA TTC CAG ACT CAA GCA GGC TGT CTT ATA

630 640
G A E H V D T S Y E C D I P I

GGA GCT GAG CAT GTC GAC ACT TCT TAT GAG TGC GAC ATT CCT ATT

650

G A G I C A S Y H T V S L L R
GGA GCT GGC ATT TGT GCT AGT TAC CAT ACA GTT TCT TTA TTA CGT

660 670

S T S Q K S I V A Y T M S L G
AGT ACT AGC CAA AAA TCT ATT GTG GCT TAT ACT ATG TCT TTA GGT

680

A D S S I A Y S N N T I A I P
GCT GAT AGT TCA ATT GCT TAC TCT AAT AAC ACC ATT GCT ATA CCT

690 700

T N F S I S I T T E V M P V S
ACT AAC TTT TCA ATT AGC ATT ACT ACA GAA GTA ATG CCT GTT TCT

710

M A K T S V D C N M Y I C G D
ATG GCT AAA ACC TCC GTA GAT TGT AAT ATG TAC ATC TGC GGA GAT

720 730

S T E C A N L L L Q Y G S F C
TCT ACT GAA TGT GCT AAT TTG CTT CTC CAA TAT GGT AGC TTT TGC

740

T Q L N R A L S G I A A E Q D
ACA CAA CTA AAT CGT GCA CTC TCA GGT ATT GCT GCT GAA CAG GAT

750 760

R N T R E V F A Q V K Q M Y K
CGC AAC ACA CGT GAA GTG TTC GCT CAA GTC AAA CAA ATG TAC AAA

770

T P T L K Y F G G F N F S Q I
ACC CCA ACT TTG AAA TAT TTT GGT GGT TTT AAT TTT TCA CAA ATA

780 790

L P D P L K P T K R S F I E D
TTA CCT GAC CCT CTA AAG CCA ACT AAG AGG TCT TTT ATT GAG GAC

800

L L F N K V T L A D A G F M K
TTG CTC TTT AAT AAG GTG ACA CTC GCT GAT GCT GGC TTC ATG AAG

810 820

Q Y G E C L G D I N A R D L I
CAA TAT GGC GAA TGC CTA GGT GAT ATT AAT GCT AGG GAC CTC ATT

830

C A Q K F N G L T V L P P L L
TGT GCG CAG AAG TTC AAT GGA CTT ACA GTG TTG CCA CCT CTG CTC

840 850

T D D M I A A Y T A A L V S G
ACT GAT GAT ATG ATT GCT GCC TAC ACT GCT GCT CTA GTT AGT GGT

860

T A T A G W T F G A G A A L Q
ACT GCC ACT GCT GGA TGG ACA TTT GGT GCT GGC GCT GCT CTT CAA

870 880

```
 I   P   F   A   M   Q   M   A   Y   R   F   N   G   I   G
ATA CCT TTT GCT ATG CAA ATG GCA TAT AGG TTC AAT GGC ATT GGA

                                890
 V   T   Q   N   V   L   Y   E   N   Q   K   Q   I   A   N
GTT ACC CAA AAT GTT CTC TAT GAG AAC CAA AAA CAA ATC GCC AAC

                900                                    910
 Q   F   N   K   A   I   S   Q   I   Q   E   S   L   T   T
CAA TTT AAC AAG GCG ATT AGT CAA ATT CAA GAA TCA CTT ACA ACA

                                920
 T   S   T   A   L   G   K   L   Q   D   V   V   N   Q   N
ACA TCA ACT GCA TTG GGC AAG CTG CAA GAC GTT GTT AAC CAG AAT

                930                                    940
 A   Q   A   L   N   T   L   V   K   Q   L   S   S   N   F
GCT CAA GCA TTA AAC ACA CTT GTT AAA CAA CTT AGC TCT AAT TTT

                                950
 G   A   I   S   S   V   L   N   D   I   L   S   R   L   D
GGT GCA ATT TCA AGT GTG CTA AAT GAT ATC CTT TCG CGA CTT GAT

                960                                    970
 K   V   E   A   E   V   Q   I   D   R   L   I   T   G   R
AAA GTC GAG GCG GAG GTA CAA ATT GAC AGG TTA ATT ACA GGC AGA

                                980
 L   Q   S   L   Q   T   Y   V   T   Q   Q   L   I   R   A
CTT CAA AGC CTT CAA ACC TAT GTA ACA CAA CAA CTA ATC AGG GCT

                990                                    1000
 A   E   I   R   A   S   A   N   L   A   A   T   K   M   S
GCT GAA ATC AGG GCT TCT GCT AAT CTT GCT GCT ACT AAA ATG TCT

                                1010
 E   C   V   L   G   Q   S   K   R   V   D   F   C   G   K
GAG TGT GTT CTT GGA CAA TCA AAA AGA GTT GAC TTT TGT GGA AAG

                1020                                   1030
 G   Y   H   L   M   S   F   P   Q   A   A   P   H   G   V
GGC TAC CAC CTT ATG TCC TTC CCA CAA GCA GCC CCG CAT GGT GTT

                                1040
 V   F   L   H   V   T   Y   V   P   S   Q   E   R   N   F
GTC TTC CTA CAT GTC ACG TAT GTG CCA TCC CAG GAG AGG AAC TTC

                1050                                   1060
 T   T   A   P   A   I   C   H   E   G   K   A   Y   F   P
ACC ACA GCG CCA GCA ATT TGT CAT GAA GGC AAA GCA TAC TTC CCT

                                1070
 R   E   G   V   F   V   F   N   G   T   S   W   F   I   T
CGT GAA GGT GTT TTT GTG TTT AAT GGC ACT TCT TGG TTT ATT ACA

                1080                                   1090
 Q   R   N   F   F   S   P   Q   I   I   T   T   D   N   T
CAG AGG AAC TTC TTT TCT CCA CAA ATA ATT ACT ACA GAC AAT ACA

                                1100
 F   V   S   G   N   C   D   V   V   I   G   I   I   N   N
TTT GTC TCA GGA AAT TGT GAT GTC GTT ATT GGC ATC ATT AAC AAC
```

```
                       1110                                               1120
 T    V    Y    D    P    L    Q    P    E    L    D    S    F    K    E
ACA  GTT  TAT  GAT  CCT  CTG  CAA  CCT  GAG  CTT  GAC  TCA  TTC  AAA  GAA

                                                 1130
 E    L    D    K    Y    F    K    N    H    T    S    P    D    V    D
GAG  CTG  GAC  AAG  TAC  TTC  AAA  AAT  CAT  ACA  TCA  CCA  GAT  GTT  GAT

                       1140                                               1150
 F    G    D    I    S    G    I    N    A    S    V    V    N    I    Q
TTT  GGC  GAC  ATT  TCA  GGC  ATT  AAC  GCT  TCT  GTC  GTC  AAC  ATT  CAA

                                                 1160
 K    E    I    D    R    L    N    E    V    A    K    N    L    N    E
AAA  GAA  ATT  GAC  CGC  CTC  AAT  GAG  GTC  GCT  AAA  AAT  TTA  AAT  GAA

                       1170                                               1180
 S    L    I    D    L    Q    E    L    G    K    Y    E    Q    Y    I
TCA  CTC  ATT  GAC  CTT  CAA  GAA  TTG  GGA  AAA  TAT  GAG  CAA  TAT  ATT

             1183
 K    W    P    OC                      SEQ ID NO: 9801
AAA  TGG  CCT  TAA  TGAGTCGAC           SEQ ID NO: 9802
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Translated Mol. Weight = 131315.20

FIGURE 67

FIGURE 67A

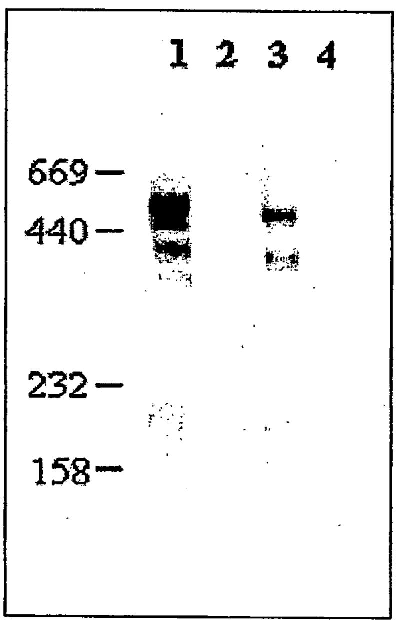

FIGURE 67B

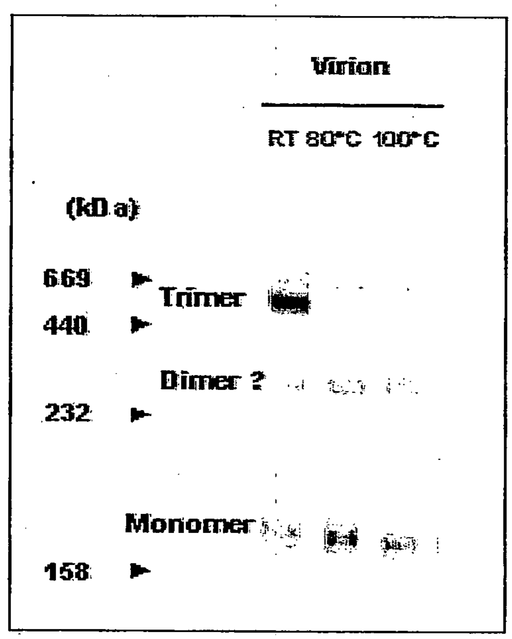

FIGURE 68

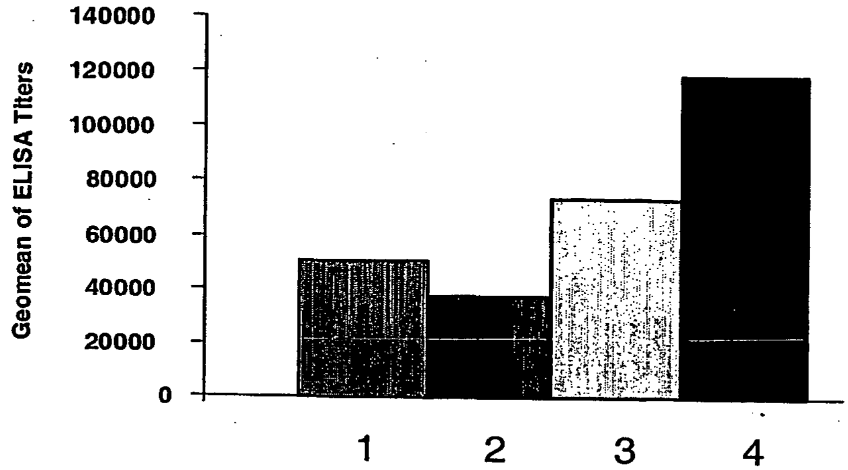

FIGURE 69

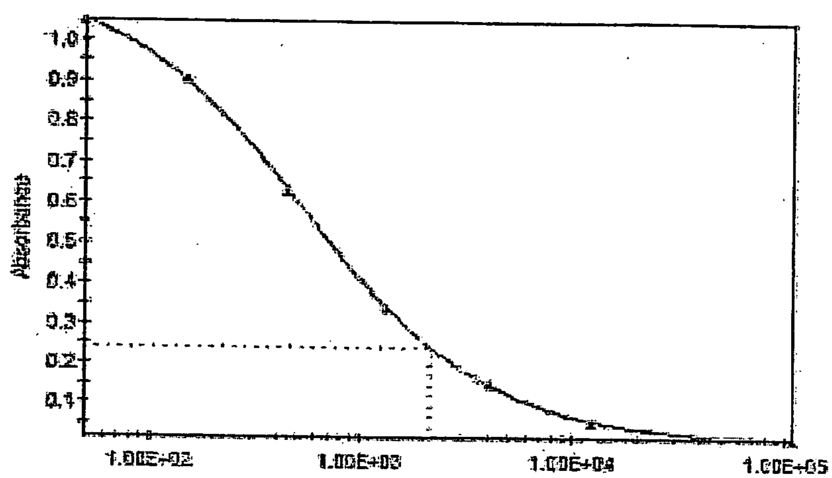

FIGURE 70

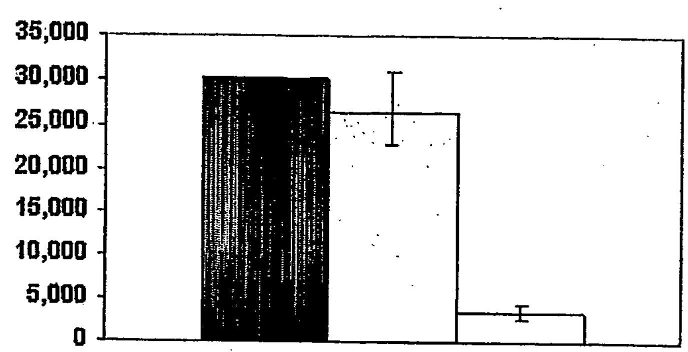

FIGURE 71

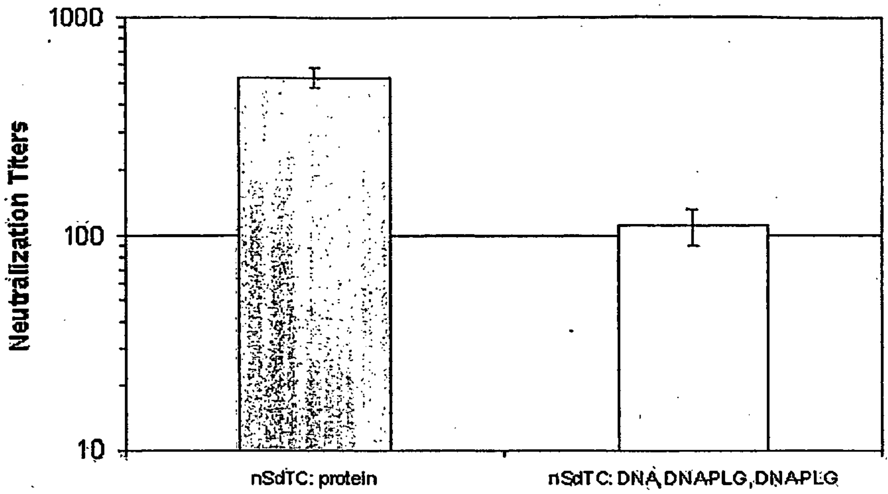

FIGURE 72

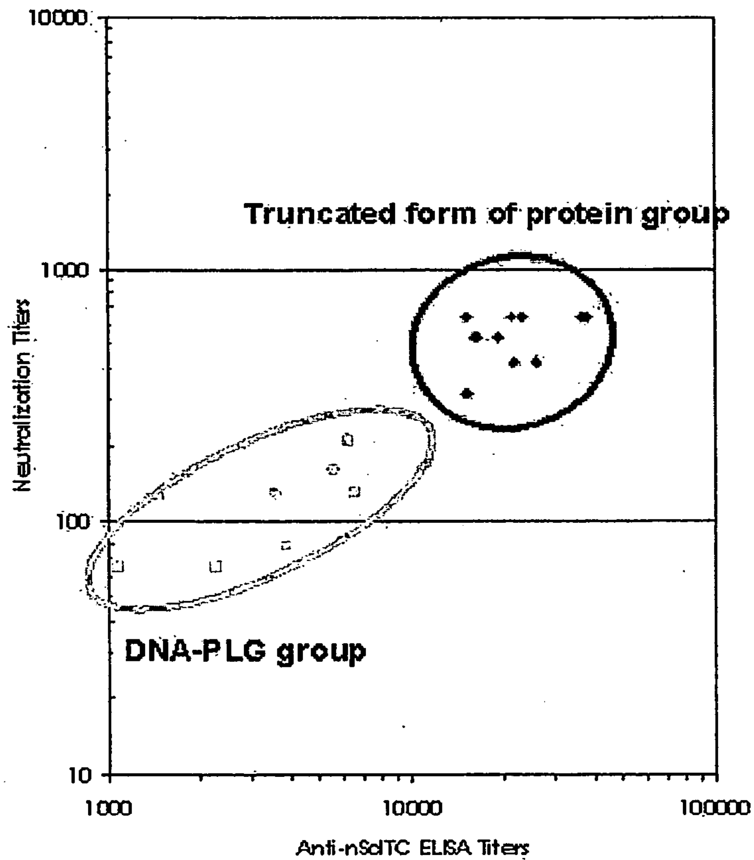

FIGURE 73

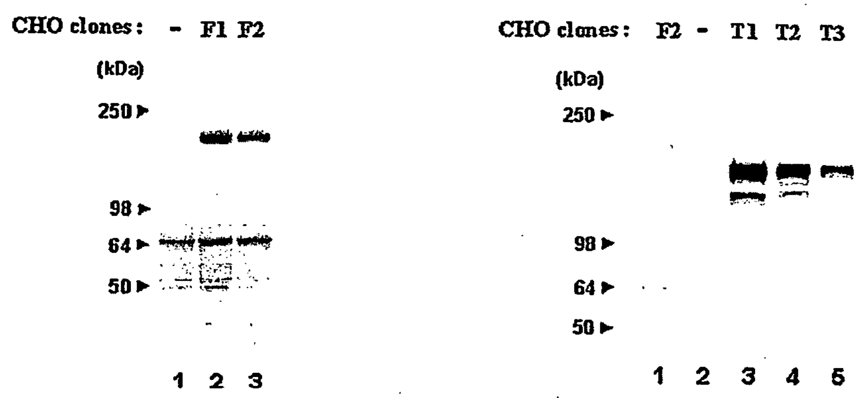

FIGURE 74

FIGURE 74A

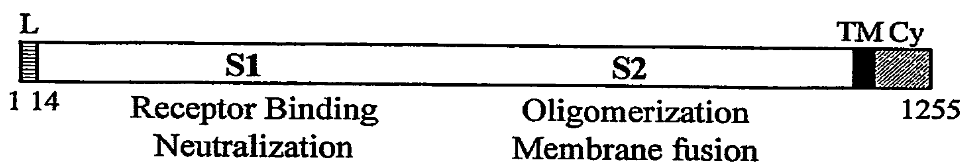

FIGURE 74B

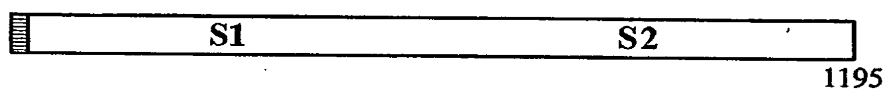

FIGURE 75

FIGURE 75A

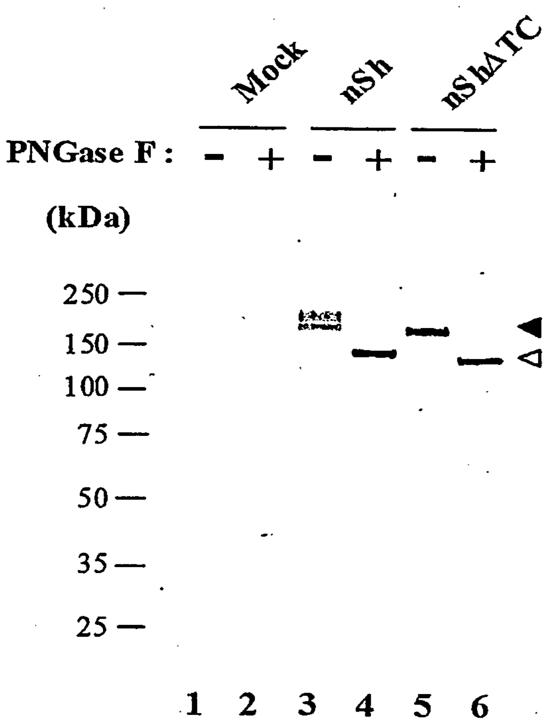

FIGURE 75B

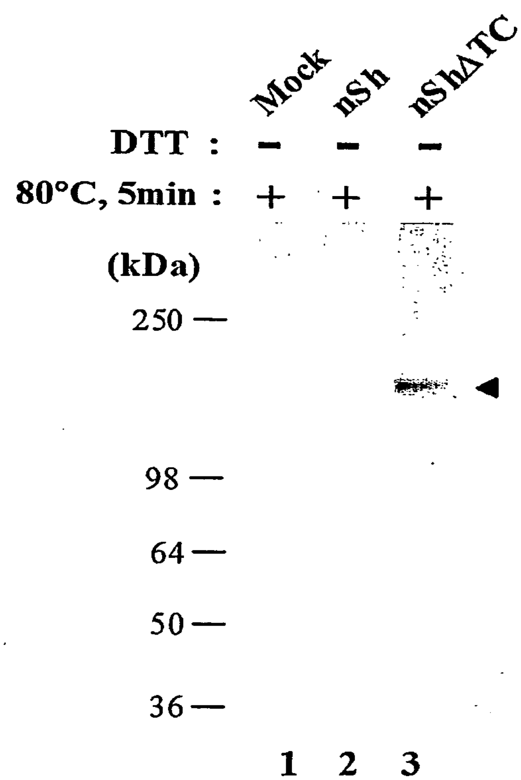

FIGURE 76

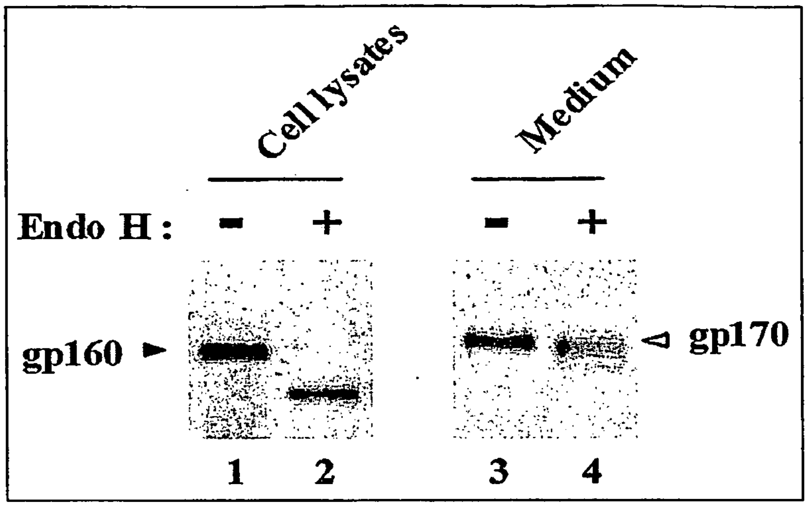

FIGURE 77

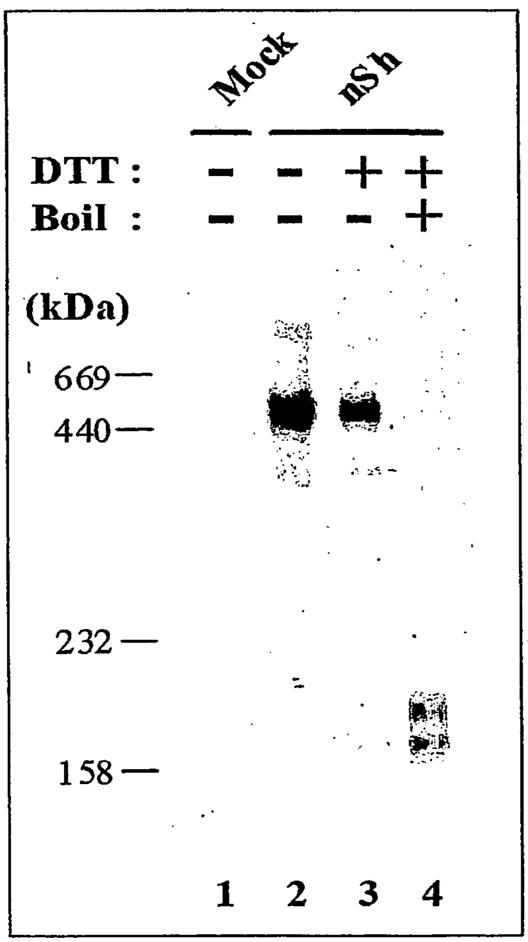

FIGURE 78

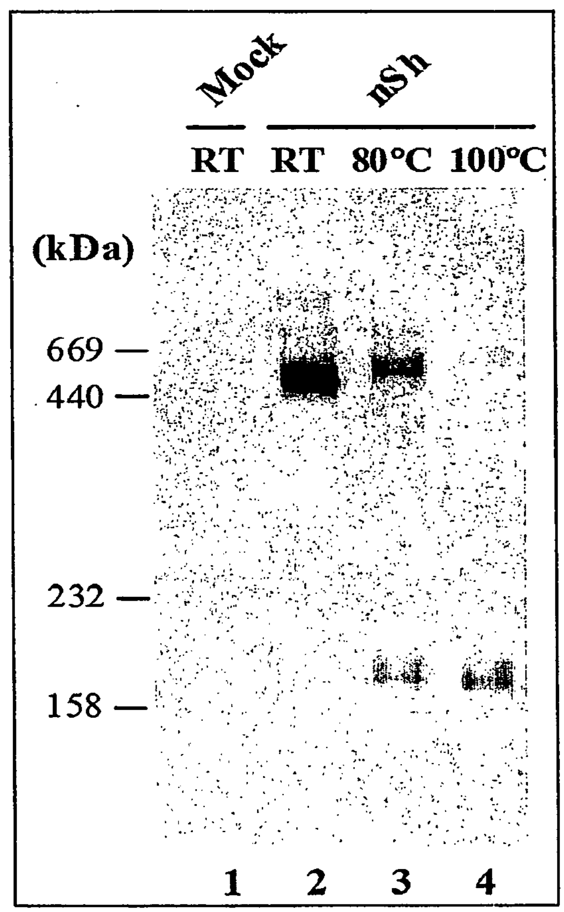

FIGURE 79

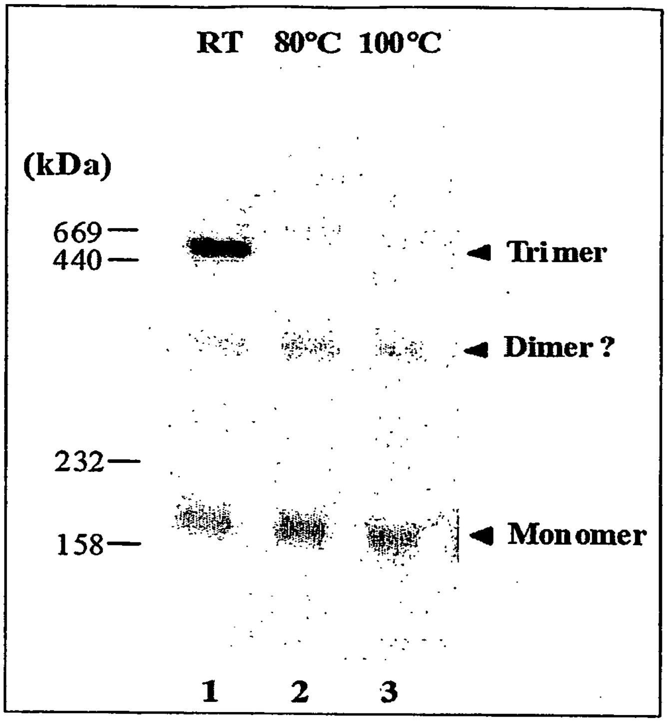

FIGURE 80

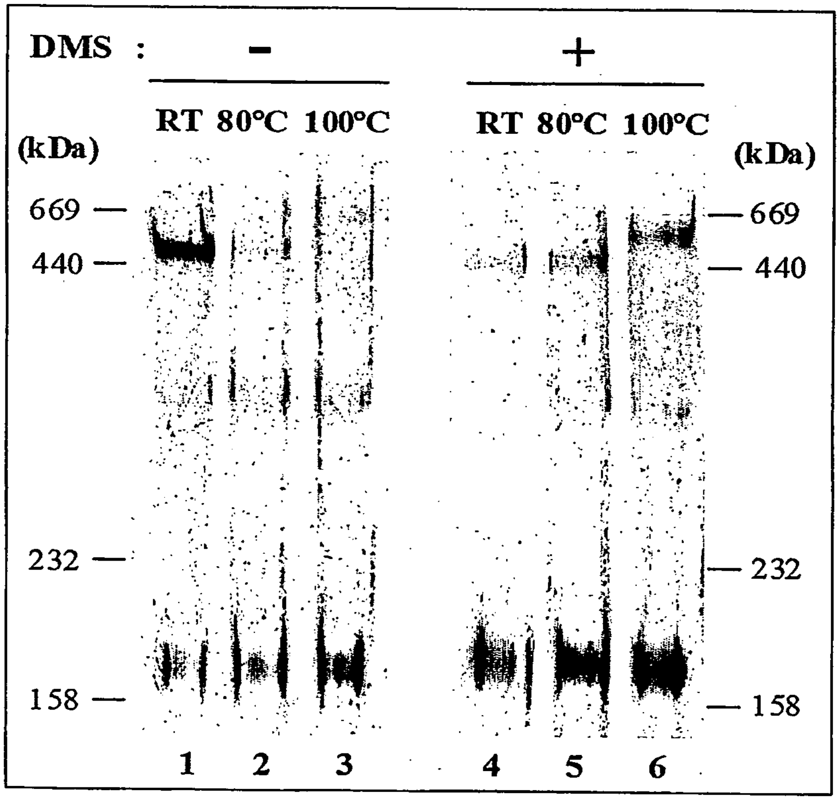

FIGURE 81

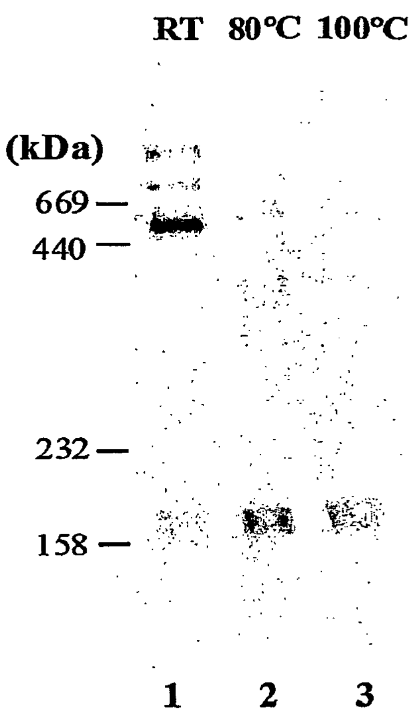

FIGURE 82

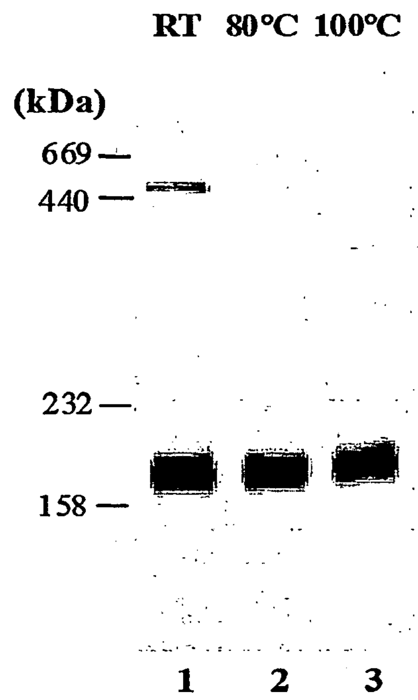

FIGURE 83

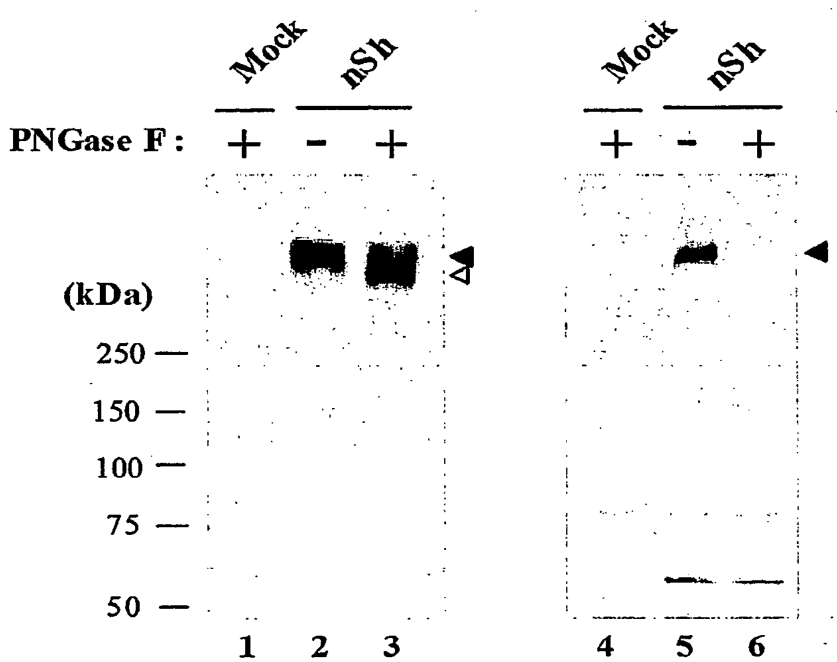

FIGURE 84

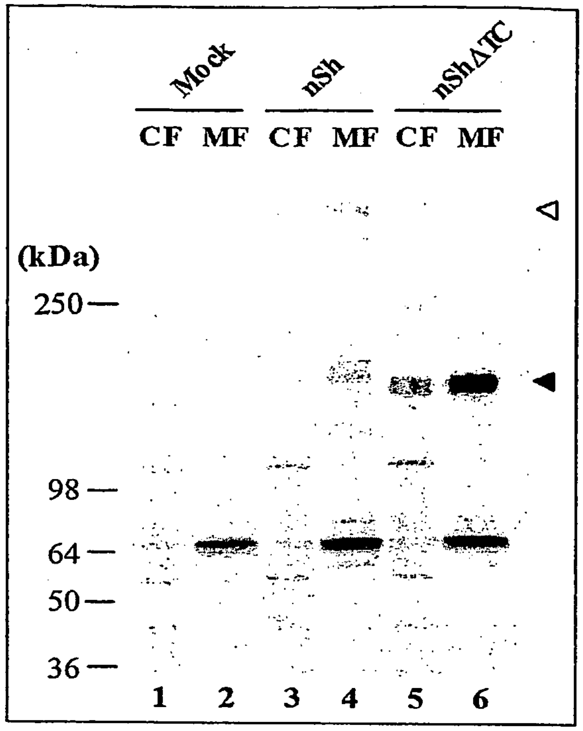

FIGURE 85

FIGURE 85A **FIGURE 85B** **FIGURE 85C**

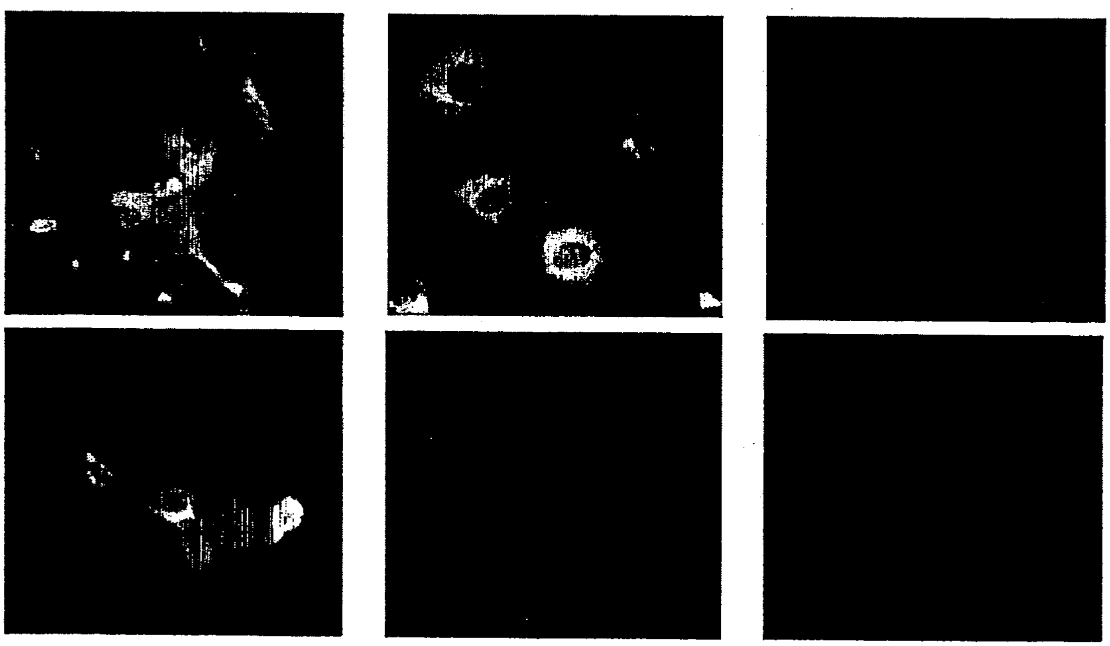

FIGURE 85D **FIGURE 85E** **FIGURE 85F**

FIGURE 86

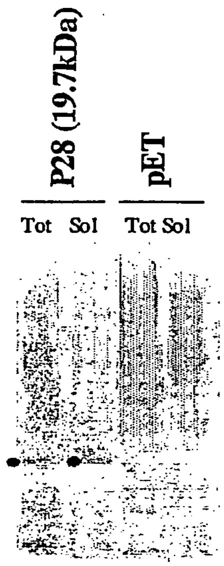

FIGURE 87

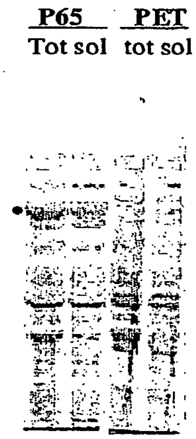

FIGURE 88

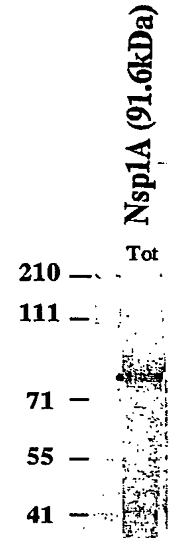

FIGURE 89

FIGURE 90

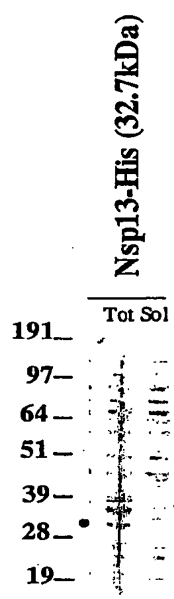

FIGURE 91

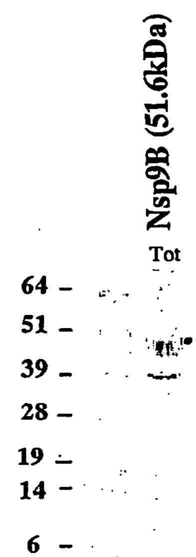

FIGURE 92

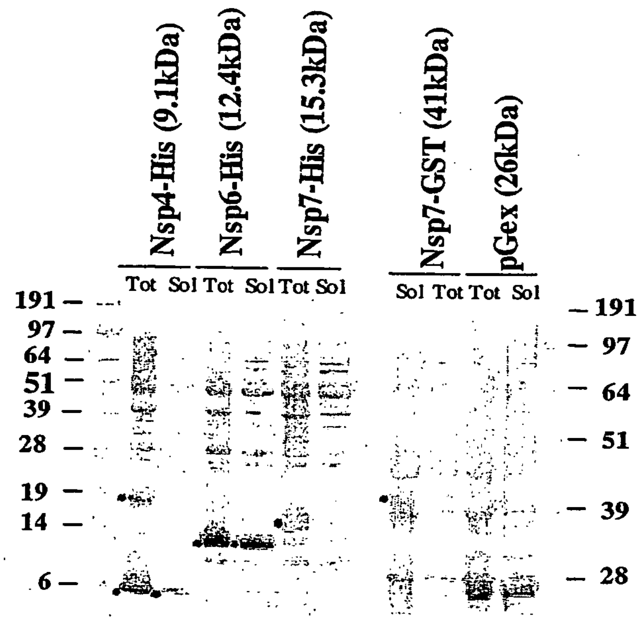

FIGURE 93

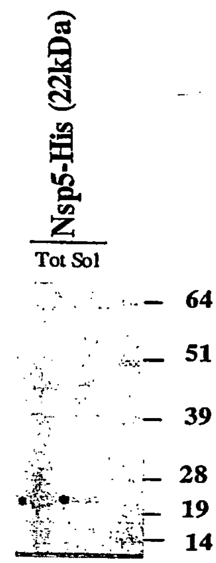

FIGURE 94

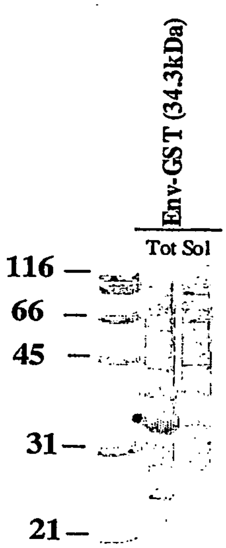

FIGURE 95

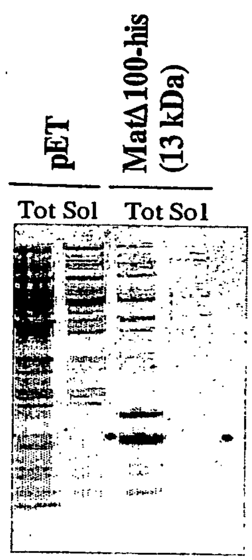

FIGURE 96

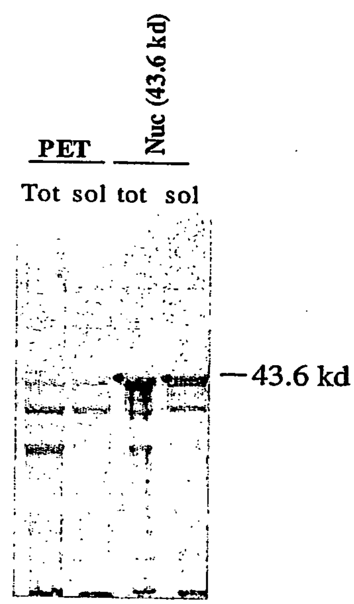

FIGURE 97

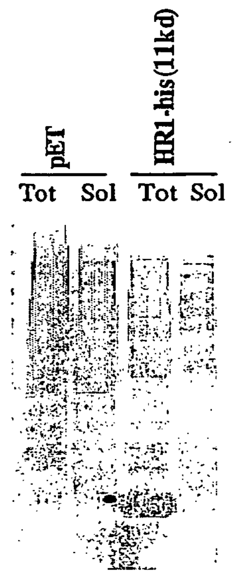

FIGURE 98

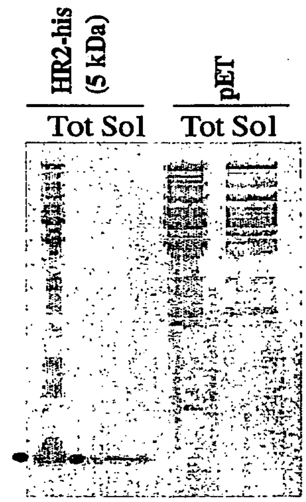

FIGURE 99

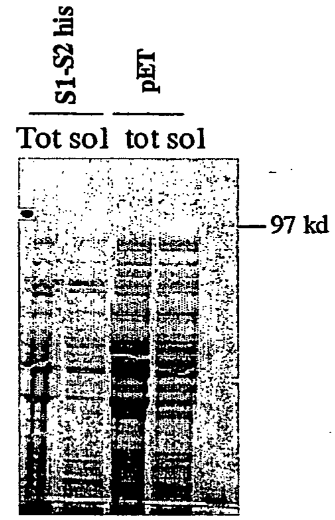

FIGURE 100

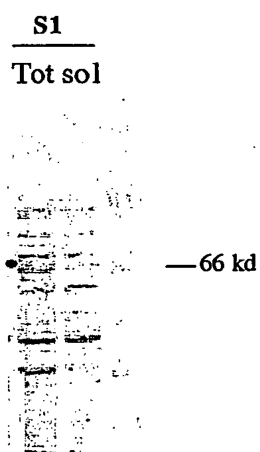

FIGURE 101

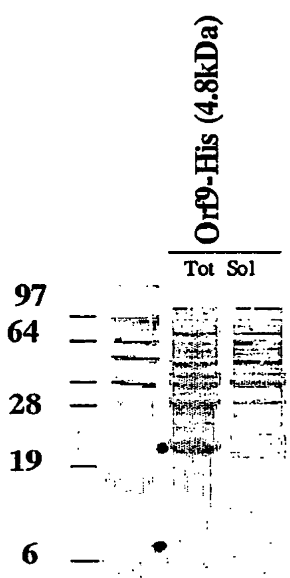

FIGURE 102

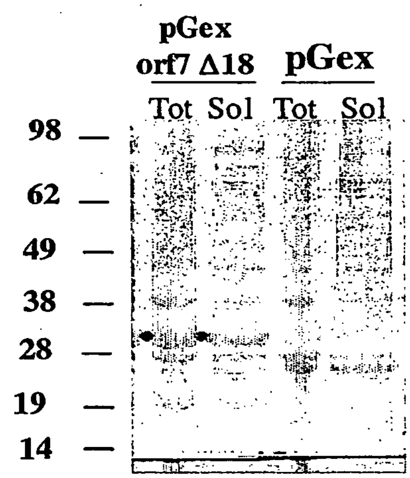

FIGURE 103

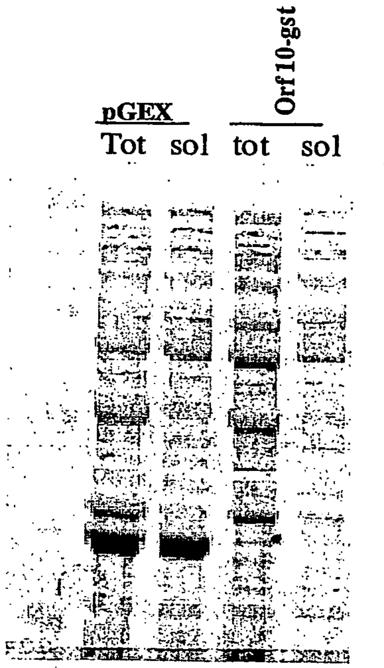

FIGURE 104

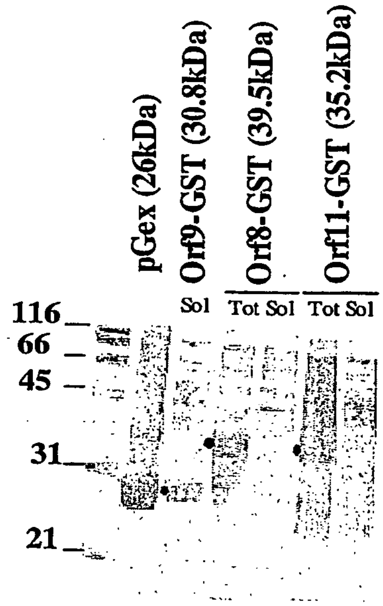

FIGURE 105

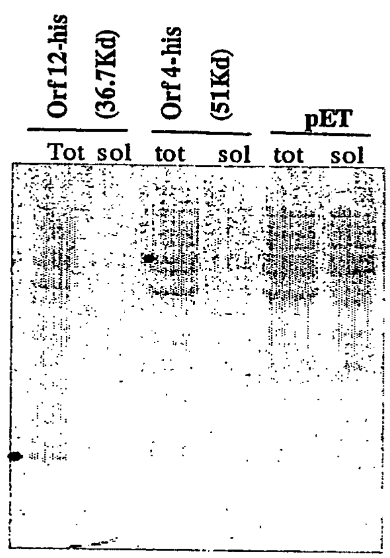

FIGURE 106

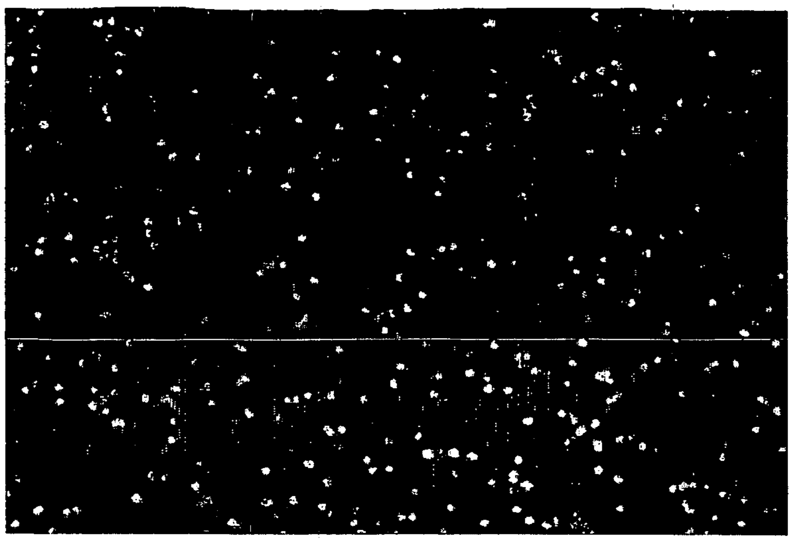

FIGURE 107

FIGURE 107A

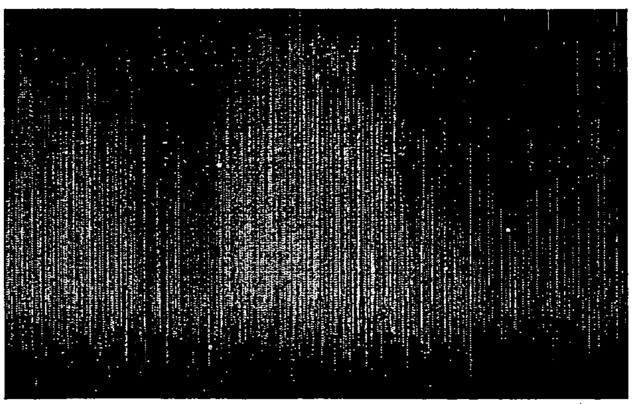

FIGURE 107B

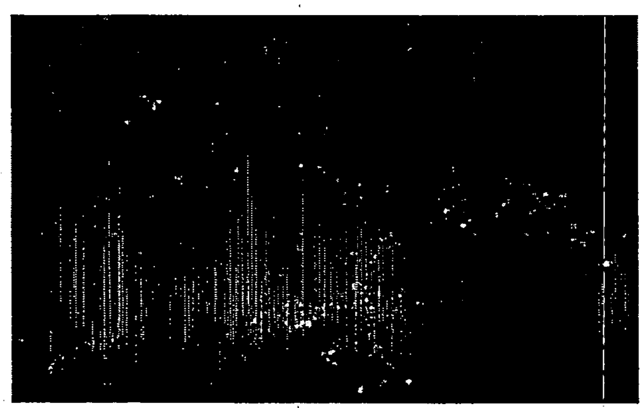

FIGURE 108

FIGURE 108A

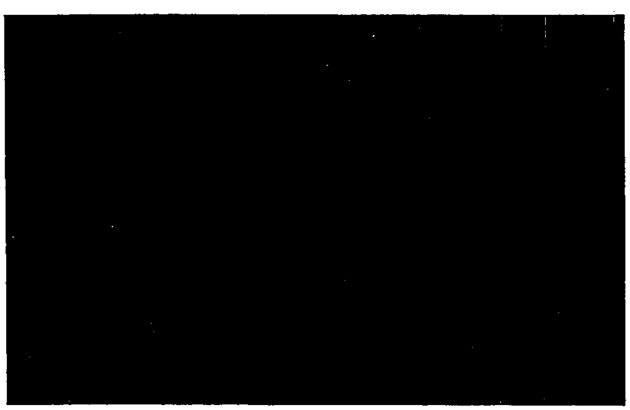

FIGURE 108B

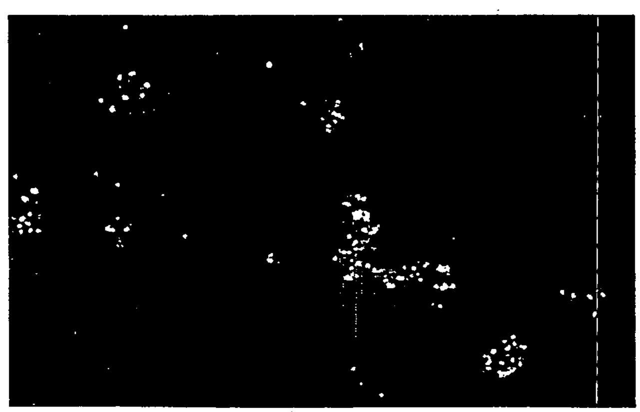

FIGURE 109

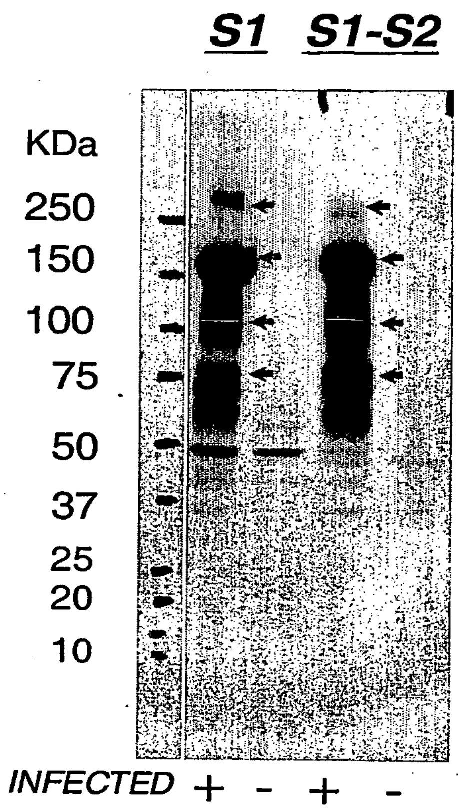

FIGURE 110

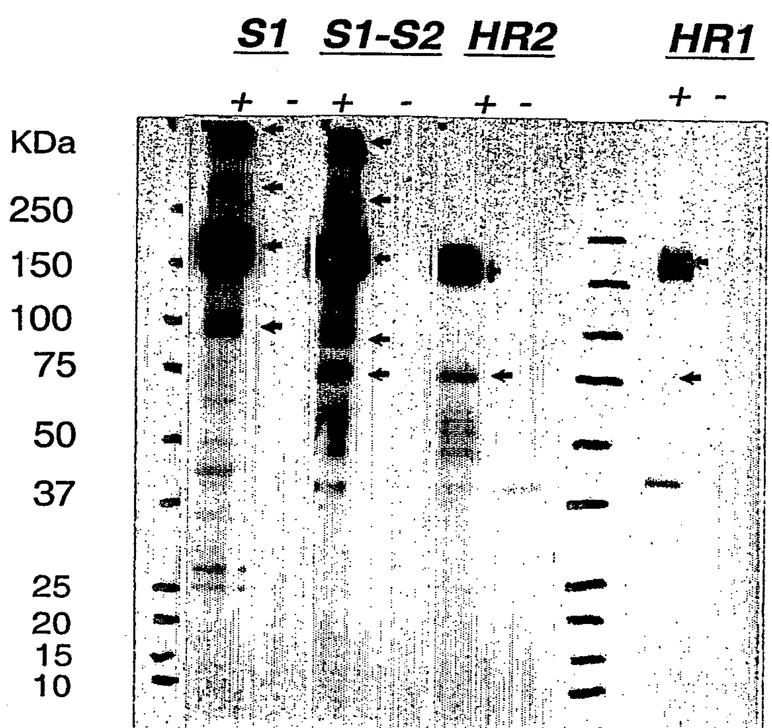

FIGURE 111

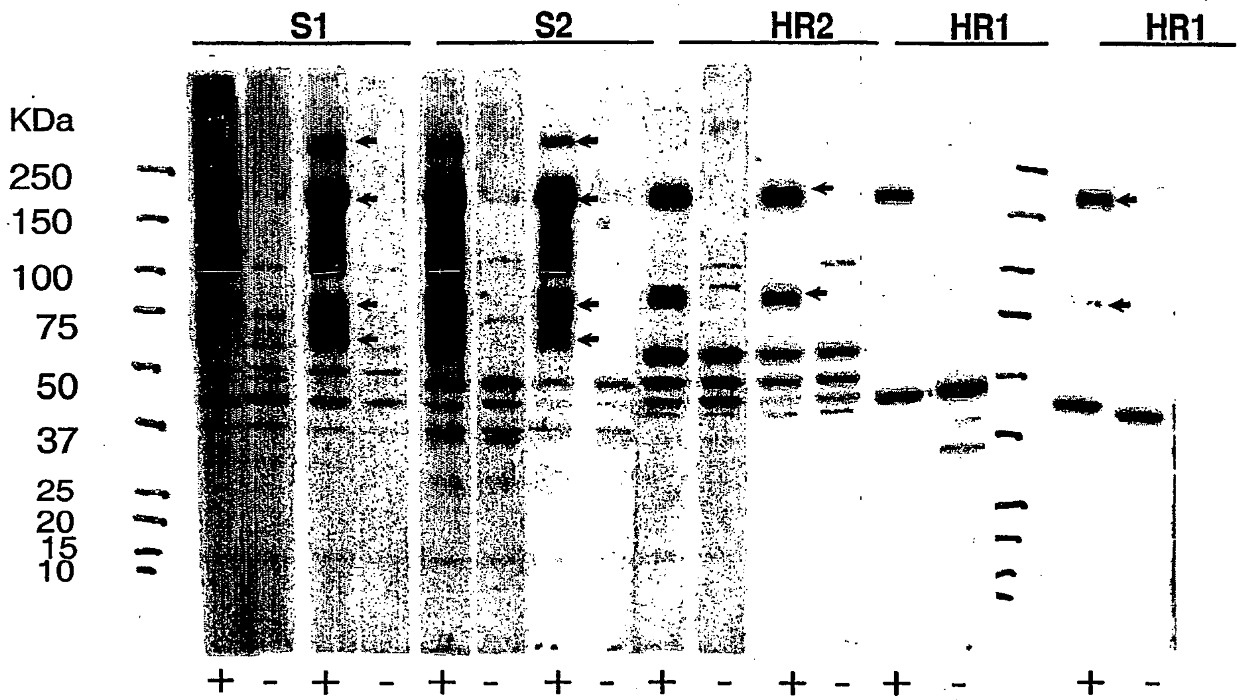

FIGURE 112

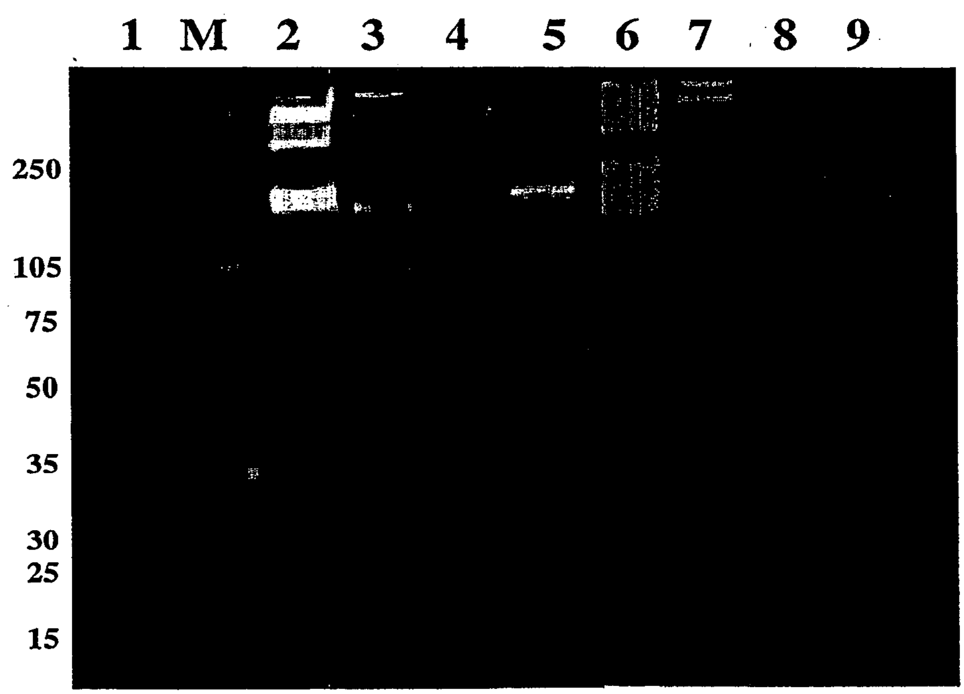

FIGURE 113

5'3' Frame 1

PKDMTYVDSSL-WVS-ITKSMVTLICLSPAKKLFVTFVRGLALM-RAVMQLEMLWVLTYL
SS-DFLQVLT--LYRLVMLTLKITQNSPELMHKPPPVSSLNILYHSCIKACPGM-CVLR-
YKCSVIH-KDCQTESCSSFGRMALSLHQ-STLSRLDLKERVVCVTNVQLAFLLHQILMPA
GIILWVLTMSITHL-LMFSSGGFTGNLSE-P-PTLPGTWKCTCGLVVML

5'3' Frame 2

QRT-PT-THLYDGFQNELPSQWLP-YVYHPRRSYSSRSCVDWL-CRGLSCN-RCCGY-PT
SPARIFYRC-LSSCTDWLC-H-K-HKIHQS-CTNLHQ-AV-TSYTTHV-RLALECSAY-D
STNAQ-YTERIVRQSRVRPLGAWL-AYINEVLCQDWT-KNVLSV-QTCNLLFYFIRYLCL
LESFCGF-LCL-PIYD-CSAVGALRVTFQSNHDQHCQVHGNAHVG-L-C

5'3' Frame 3

KGHDLRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLP
LQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLMYKGLPWNVVRIKI
VQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYAC
WNHSVGFDYVYNPFMIDVQQWGLYG-PFRVTMTNIARYMEMHMWASCDA

3'5' Frame 1

-HHN-PTCAFPCTWQCWSWLL-KVTRKAPTAEHQS-MGYRHSQNPQNDSSRHKYLMK-KS
KLHVCHTDNTFFQVQS-QSTSLM-AQSHAPKGRTRLCLTILSVYH-AFVLS-YALHSRAS
LYT-VV-DV-TAHWWRFVH-LW-ILCYFQCQHNQSVQLLS-HL-KILAGEVG-YPQHL-L
HDSPLHQSQSTHERDE-LLRG--TY-GNH-LGNSF-NPS-R-VYVGHVLW

3'5' Frame 2

SITTSPHVHFHVPGNVGHGYSERLPVKPPLLNINHKWVIDIVKTHRMIPAGISI--SRKA
SCTFVTQTTRSFRSNLDKVLH-CKLKAMRPKDEHDSV-QSFQCITEHLYYLNTHYIPGQA
FIHEWYKMFKLLTGGGLCINSGEFCVIFSVNITSRYSY-VNTCRKS-LER-VSTHSISSC
MTALYIKANPRTNVTNSFFAGDKHIRVTIDLVIHFETHHRDEST-VMSF

3'5' Frame 3

ASQLAHMCISMYLAMLVMVTLKGYP-SPHC-TSIINGL-T-SKPTE-FQQA-VSDEVEKQ
VARLSHRQHVLSGPILTKYFIDVSSKPCAQRTNTTLSDNPFSVSLSICTILIRTTFQGKP
LYMSGIRCLNCSLVEVCALTLVNFVLFSVST-PVGTATKLTPVENPSWRGRLVPTASLVA
-QPSTSKPIHART-RIASSRVINILG-PLTW-FILKPIIEMSLRRSCPL

FIGURE 114

5'3' Frame 1

YRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQLG
FSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLI

5'3' Frame 2

TVDSSL-WVSK-ITKSMVTLICLSPAKKLFVTFVRGLALM-RAVMQLEMLWVLTYLSS-D
FLQVLT--LYRLVMLTLKITQNSPELMQNLHQVTSLNILY

5'3' Frame 3

P-THLYDGFQNELPSQWLP-YVYHPRRSYSSRSCVDWL-CRGLSCN-RCCGY-PTSPARI
FYRC-LSSCTDWLC-H-K-HRIHQS-CKTSTR-PV-TSYT

3'5' Frame 1

GIRCLNWSPGGGFALTLVNSVLFSVST-PVGTATKLTPVENPSWRGRLVPTASLVA-QPS
TSKPIHART-RIASSRVINILG-PLTW-FILKPIIEMSLR

3'5' Frame 2

V-DV-TGHLVEVLH-LW-ILCYFQCQHNQSVQLLS-HL-KILAGEVG-YPQHL-LHDSPL
HQSQSTHERDE-LLRG--TY-GNH-LGNSF-NPS-R-VYG

3'5' Frame 3

YKMFKLVTWWRFCINSGEFCVIFSVNITSRYSY-VNTCRKS-LER-VSTHSISSCMTALY
IKANPRTNVTNSFFAGDKHIRVTIDLVIHFETHHRDESTV

FIGURE 115

```
                                                         Section 151
(5851) 5851      5860         5870              5889
(5675) LTNYELSVINARIRAKHYVYIGDPAQLPAPRVLLSKGTL
(5247) LTNYELSFINGKINYCYVVYVGDEAQLPAPRILLNG-SI
(5762) LTNYELSVINSRVSAKHYVYIGDPAQLPAPRVLLNEGTL
   (1) ---------------------------------------
(5851) LTNYELSVINARI AKHYVYIGDPAQLPAPRVLLNKGTL
                                                         Section 152
(5890) 5890      5900         5910              5928
(5714) EPKYFNTVTKLMCCLGPDIFLGTCYRCPKEIVDTVSAIV
(5285) SPKDYNVVTNLMVCVKPDTFLAKCYRCPKEIVDTVSTLV
(5801) EPRYFNSVTKLMCCLGPDIFLGTCYRCPKEIVDTVSALV
   (1) ---------------------------------------
(5890) EPKYFNSVTKLMCCLGPDIFLGTCYRCPKEIVDTVSALV
                                                         Section 153
(5929) 5929        5940       5950                5967
(5753) YENKLKAMNESSSLCFKVYYKG---VTTHESSSAVNMQQ
(5324) YDGKFIANNPESRECFKVIVNNGNSDVGHESGSAYNTTQ
(5840) YDNKLKAKNDNSSMCFKVYYKG---QTTHESSSAVNMQQ
   (1) ---------------------------------------
(5929) YDNKLKAKND SSLCFKVYYKG    TTHESSSAVNMQQ
                                                         Section 154
(5968) 5968        5980       5990                6006
(5789) IYLINKFLKRNPLWHKAVFISPYNSQNFAAKRVLGLQTQ
(5363) IEFYKDEVCRNKQWREAIFISPYNAMNQRAYRMLGLNVQ
(5876) IHLISKFLKANPSWSNAVFISPYNSQNYVAKRVLGLQTQ
   (1) ---------------------------------------
(5968) IHLI KFLKANP W  AVFISPYNSQNF AKRVLGLQTQ
                                                         Section 155
(6007) 6007         6020       6030               6045
(5828) TVDSAQGSEYDYVIYSQTAETAHSVNVNRFNVAITRAKK
(5402) TVDSSQGSEYDYVIFCVTADSQHALNINRFNVALTRAKR
(5915) TVDSAQGSEYDFVIYSQTAETAHSVNVNRFNVAITRAKK
   (1) ---------------------------------------
(6007) TVDSAQGSEYDYVIYSQTAETAHSVNVNRFNVAITRAKK
                                                         Section 156
(6046) 6046         6060       6070               6084
(5867) GILCVMSNMQLFEALQFTTLTLDKVPQAVETRVQCSTNL
(5441) GILVVMRQRLELYSALKFTELDSETS-----LQG--TCL
(5954) GILCVMSSMQLFESLNFTTLTLDKIN---NPRLQCTTNL
   (1) ---------------------------------------
(6046) GILCVMSNMQLFESLNFTTLTLDKI      RLQCSTNL
```

START

FIGURE 115 (contd.)

Section 157

(6085) 6085 6090 6100 6110 6123
(5906) FKDCSKSYSGYHPAHAPSFLAVDDKYKATGDLAVCLGIG
(5473) EKICDKEFSGVHPAVAVTTKALAATYKVNDELAPLVNVE
(5990) DKDCSRSYVGYHPAHAPSFLAVDDKYKVGGDLAVCLNVA
(1) ---------------------------------------
(6085) FKDCSKSYSGYHPAHAPSFLAVDDKYKV GDLAVCLNVA

Section 158

(6124) 6124 6130 6140 6150 6162
(5945) D-SAVTTYSR[illegible]
(5512) AGSETTTKH[illegible]
(6029) D-SAVTYSR[illegible]
(1) --KGHDLRR[illegible]
(6124) D SAVTYSRLISLMGFKLDVTLDGYCNLFITRDEAIKRV

Section 159

(6163) 6163 6170 6180 6190 6201
(5983) [illegible]
(5551) [illegible]
(6067) [illegible]
(38) [illegible]
(6163) RANVGFDVEGAHATRDSIGTNLPLQLGFSTGIDFVVEPT

Section 160

(6202) 6202 6210 6220 6230 6240
(6022) [illegible]
(5590) [illegible]
(6106) [illegible]
(77) [illegible]
(6202) GLVDTRDGY FKKVNAKAPPGEQFKHLIPLMSRGQPWDV

Section 161

(6241) 6241 6250 6260 6279
(6061) [illegible]
(5629) [illegible]
(6145) [illegible]
(116) [illegible]
(6241) VRPRIVQMLADHL DLSDCVVLVTWAHGFELTCLRYFVK

Section 162

(6280) 6280 6290 6300 6318
(6100) [illegible]
(5668) [illegible]
(6184) [illegible]
(155) [illegible]
(6280) IGREISCCVCTKRATCFNSRTGYYACWRHSVGFDYLYNP

FIGURE 115 (contd.)

```
                                                        Section 163
(6319) 6319       6330          6340                    6357
(6139) LIVDIGQGGYIGSLSSNHDLYCSVHKGAHVASSDAIMTR
(5706) LLVLI   GYSGNLQFNHDLHCNVHGHA-VASVDAIMTR
(6223) LIVLI  G YLGSLTSNHDPICSVHKGA VASSDAIMTR
 (194) TMIDVQQ LYG-PFRVTMTHIARYMENLMVASCDA
(6319) LIVDIQQNGYSGSLSSNHDLHCSVHKGAHVASSDAIMTR
                                                        Section 164
(6358) 6358       6370         6380                     6396
(6178) CLAVYDCFCNNINWNVEYPIISNELSINTSCRVLQRVKL
(5745) CLAINNAFGQDVNWDLTYPHIANEDEVNSSGRYLQRMYL
(6262) CLAVHDCFCKSVNWNLEYPIISNEVSVNTSCRLLQRVMF
 (229) ---------------------------------------
(6358) CLAVHDCFCN VNWNLEYPIISNELSVNTSCRLLQRVML
                                                        Section 165
(6397) 6397        6410         6420                     6435
(6217) KAAMLCNRYTLCYDIGNPKAIACVKD--FDFKFYDAQPI
(5784) NACVDALKVNVVYDIGNPKGIKCVRRGDVNERFYDKNPI
(6301) RAAMLCNRYDVCYDIGNPKGLACVKG--YDFKFYDASPV
 (229) ---------------------------------------
(6397) KAAMLCNRY VCYDIGNPKGIACVK  FDFKFYDANPI
                                                        Section 166
(6436) 6436         6450         6460                     6474
(6254) VKSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVV
(5823) VRNVKQFEYDYNQHKDKFADGLCMFWNCNVDCYPDNSLV
(6338) VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVV
 (229) ---------------------------------------
(6436) VKSVKQFLY YEAHKD F DGLCMFWNCNVDKYP NAVV
                                                        Section 167
(6475) 6475  6480       6490         6500                  6513
(6293) CRFDTRVLNNLNLPGCNGGSLYVNKHAFHTKPFSRAAFE
(5862) CRYDTRNLSVFNLPGCNGGSLYVNKHAFYTPKFDRISEF
(6377) CRFDTRVLNKLNLPGCNGGSLYVNKHAFHTSPFTRAAFE
 (229) ---------------------------------------
(6475) CRFDTRVLN LNLPGCNGGSLYVNKHAFHT PFSRAAFE
                                                        Section 168
(6514) 6514  6520       6530         6540                  6552
(6332) HLKPMPFFYYSDTPCVYMDGMDAKQVDYVPLKSATCITR
(5901) NLKAMPFFFYDSSPCETIQVDG-VAQDLVSLATKDCITK
(6416) NLKPMPFFYYSDTPCVYMEGMESKQVDYVPLRSATCITR
 (229) ---------------------------------------
(6514) NLKPMPFFYYSDTPCVYMDGMDAKQVDYVPLKSATCITR
```

FIGURE 115 (contd.)

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Section 169
(6553) 6553      6560        6570          6580        6591
(6371) CNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFD
(5939) CNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNLLN
(6455) CNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFD
 (229) ---------------------------------------
(6553) CNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFD

Section 170
(6592) 6592      6600        6610        6620        6630
(6410) FYNLWNTFTKLQSLENVVYNLVKTGHYTGQAGEMPCAII
(5978) PYNLWKSESALQSIDNIAYNMYKGGHYDAIAGEMPTVIT
(6494) FYNLWNTFTRLQSLENVVYNLVNAGHFDGRAGEFPCAVI
 (229) ---------------------------------------
(6592) FYNLWNTFTKLQSLENVVYNLVKAGHYDG AGEMPCAII
                   stop

Section 171
(6631) 6631      6640        6650                    6669
(6449) GDKVVAKIIKEDVVIFINNTTYPTNVAVELFAKRSIRHH
(6017) GDKVFVIDQGVEKAVFVNQTTLPTSVAFELYAKRNIKTL
(6533) GEKVIAKIQNEDVVVEKNNTIFPTNVAVELFAKRSIRPH
 (229) ---------------------------------------
(6631) GDKVIAKIQ EDVVVFINNTTFPTNVAVELFAKRSIR H

Section 172
(6670) 6670        6680        6690                  6708
(6488) PELKLFRNLNIDVCWKHVIWDYAKESIFCSNTYGVCMYT
(6056) PNNRELKGLCVDVTNGFVIWDYANQTPLYRNTVKVCAYT
(6572) PELKLFRNLNIDVCWSHVLWDYAKDSVFCSSTYKVCKYT
 (229) ---------------------------------------
(6670) PELKLFRNLNIDVCW HVIWDYAKDSIFCSNTYKVC YT

Section 173
(6709) 6709          6720        6730                  6747
(6527) DLKFIDKLNVLFDGRDNGALEAFKRSNNGVYISTTKVKS
(6095) DIEPNG--LMVLYDDRYGDYQSFLAADNAVLVSTQCYKR
(6611) DLQCIESLNVLFDGRDNGALEAFKKTRNGVYINTTKIKS
 (229) ---------------------------------------
(6709) DL  ID LNVLFDGRDNGALEAFKKA NGVYISTTKIKS

Section 174
(6748) 6748          6760        6770                  6786
(6566) LSMIKGPPRAELNGVVVDKVGDTDCVFYTAVRKEGQDVI SEQ ID NO: 10068
(6132) YSYVSIPSNLLVQNGMPLKDG-------------ANLYV SEQ ID NO: 10069
(6650) LSMIKGPQRADLNGVVVEKVGDSDVEFWTAVRKDGNDVI SEQ ID NO: 10070
 (229) --------------------------------------- SEQ ID NO: 9997/98
(6748) LSMIKGP RADLNGVVVDKVGDSD  FWFAVRKDGNDVI SEQ ID NO: 10071
```

FIGURE 116

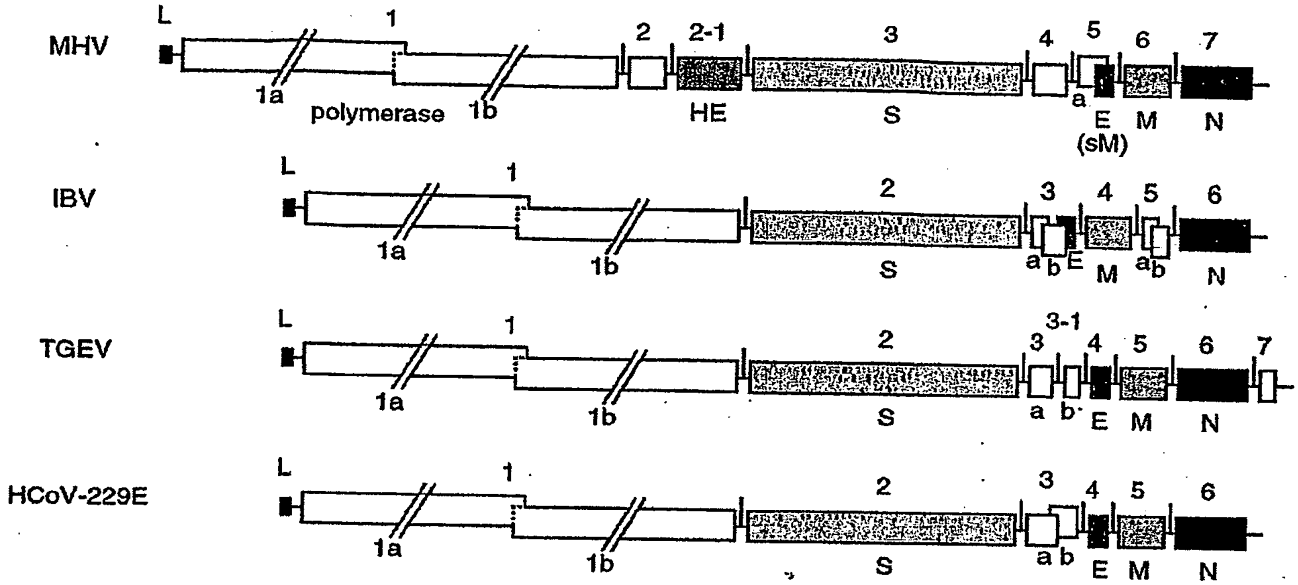

FIGURE 117

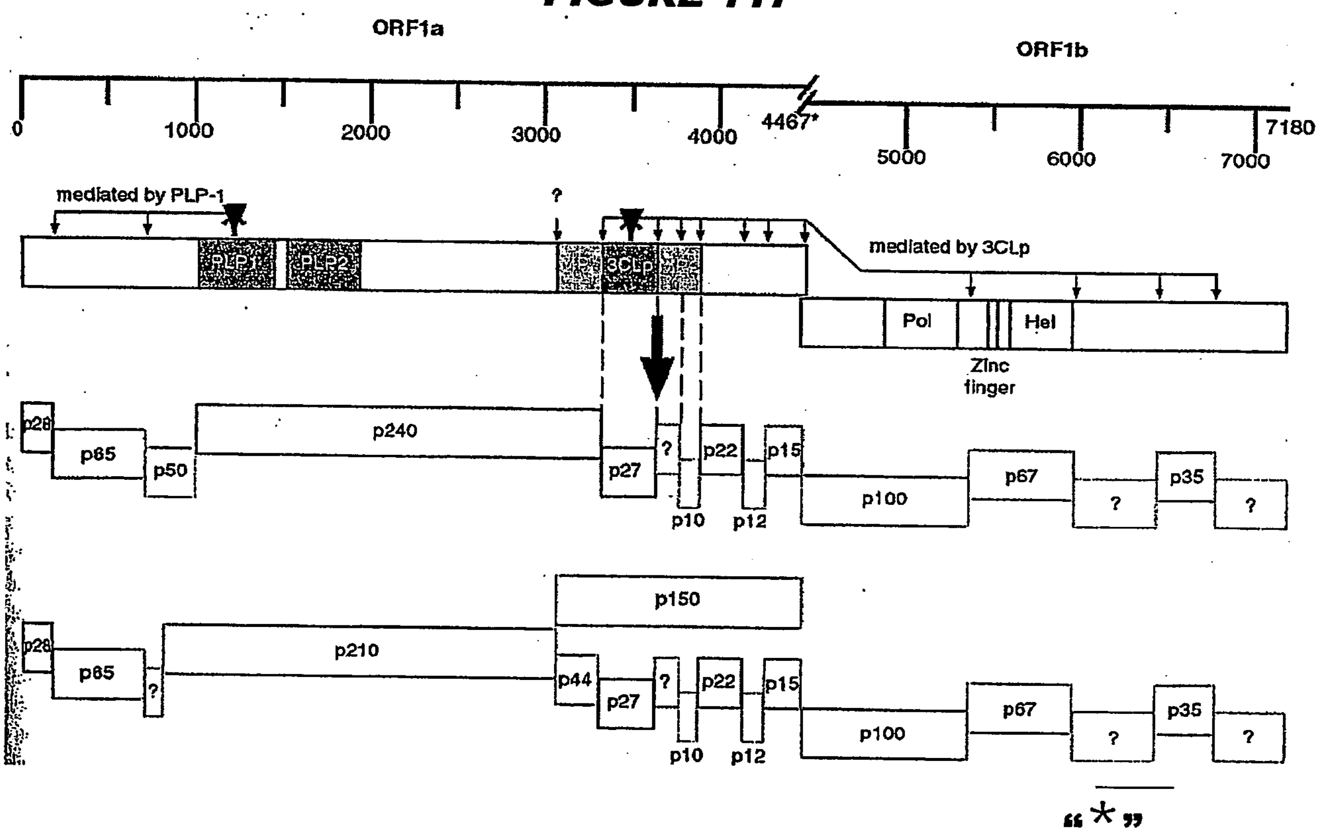

FIGURE 118

```
                                                              Section 1
(1)   1        10        20        30        40        51
(1)   -GYGILFKI NKEFSGV  H Y VTTKALAAT  VHDEL ALVNVEAG
(1)   CSTNLFFD SKSYSCY  H  PSFLAVDDK SATGDLAVCLGIGD-
(1)   CTTNLFED SRSYV Y P HAPSFLAVDDK VGGDLAVCLNVAD-KGHDL
(1)   CSTNLFED SKSYSCY P H PSFLAVDDK VGGDL VCLNVAD-
(1)   CSTNLFKDCSKSYSGYHPAHAPSFLAVDDKYKVGGDLAVCLNVAD KGHDL
                                                              Section 2
(52)  52       60        70        80        90        102
(51)  RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
(51)  RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
(51)  RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
(51)  RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
(52)  RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
                                                              Section 3
(103) 103      110       120       130       140       153
(102) TNLPLQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLM
(102) TNLPLQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLM
(102) TNLPLQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLM
(102) TNLPLQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLM
(103) TNLPLQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLM
                                                              Section 4
(154) 154      160       170       180       190       204
(153) YKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPE
(153) YKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPE
(153) YKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPE
(153) YKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPE
(154) YKGLPWNVVRIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPE
                                                              Section 5
(205) 205  210       220       230       240           255
(204) RTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGLYGNLQF
(204) RTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGLYGSLSS
(204) RTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGLYGSLTS
(204) RTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGLYGSLSS
(205) RTCCLCDKRATCFSTSSDTYACWNHSVGFDYVYNPFMIDVQQWGLYGSLSS
                                                              Section 6
(256) 256           270       280       290               306
(255) NHDLHSN HGHA IV SVDAIMT CL IMNAF QDV WDLT EHLALFDEVN
(255) NHDLYCSVHKGAHVASSDAIMT CLAVYDC  NNIT NVEL FII SELLSIN
(255) NHDPICSVHKGAHVASSDAIM RCLAVHDC  KSVN NLE PITSNFVSVN
(255) NHDLHCSVHKGAHVASSDAIMTRCLAVHDCFCNSVN NLEYPIISNELSVN
(256) NHDLHCSVHKGAHVASSDAIMTRCLAVHDCFCNSVNWNLEYPIISNELSVN
```

FIGURE 118 (contd.)

```
                                                            Section 7
(307) 307           320          330          340                357
(306) S[illegible]Y[illegible]EMYLM[illegible]VDALKVNVV[illegible]GIK[illegible]RRGDVN[illegible]R[illegible]KN[illegible]I[illegible]
(306) T[illegible]V[illegible]VMLK[illegible]AMLCNR[illegible]TLC[illegible]DI[illegible]AIAC[illegible]K--DFD[illegible]K[illegible]LAQ[illegible]I[illegible]
(306) T[illegible]LL[illegible]VMER[illegible]AMLCNRYDVC[illegible]DIG[illegible]GLA[illegible]K--[illegible]YD[illegible]KF[illegible]DAS[illegible]V[illegible]
(306) T[illegible]L[illegible]VMLK[illegible]AMLCNR[illegible]TVC[illegible]GIAC[illegible]K--DFDTK[illegible]LANPI[illegible]
(307) TSCRLLQRVMLKAAMLCNRYTVCYDIGNPKGIACVK  DFDFKFYDANPIV
                                                            Section 8
(358) 358           370          380          390                408
(357) RNVKQFEYDYNQHKDKFADGLCMFWNCNVDCYRDNSLVCRYDPRNLSVFNL
(355) KSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNNLNL
(355) KSVKQFVYKYEAHKDQLLDGLCMFWNCNVDKYPANAVVCRFDTRVLNKLNL
(355) KSVKQFLYSYEAHKDSFKDGLCMFWNCNVDKYPANAVVCRFDTRVLN-LNL
(358) KSVKQFLYSYEAHKDSFKDGLCMFWNCNVDKYPANAVVCRFDTRVLN LNL
                                                            Section 9
(409) 409           420          430          440                459
(408) [illegible]FY[illegible]PK[illegible]IS[illegible]N[illegible]A[illegible]FF[illegible]S[illegible]ETIQ-VDGVA
(406) PGCH[illegible]SL[illegible]VN[illegible]HA[illegible]H[illegible]KP[illegible]S[illegible]AAFEHL[illegible]P[illegible]F[illegible]Y-SDT[illegible]VYMDGMDAKQ
(406) PG[illegible]GGSLY[illegible]N[illegible]H[illegible]SP[illegible]AA[illegible]EN[illegible]P[illegible]F[illegible]Y-SDTP[illegible]VYME[illegible]GMESKQ
(405) P[illegible]H[illegible]KPFS[illegible]AA[illegible]EN[illegible]F[illegible]Y[illegible]SDT[illegible]V[illegible]D[illegible]DAKQ
(409) PGCNGGSLYVNKHAFHTKPFSRAAFENLKPMPFFYYSDTPCVYMDGMDAKQ
                                                            Section 10
(460) 460           470          480          490          500   510
(458) Q[illegible]LY[illegible]ATKD[illegible]K[illegible]CK[illegible]AQM[illegible]ATFVT[illegible]T[illegible]
(457) V[illegible]Y[illegible]P[illegible]KSAT[illegible]LIR[illegible]L[illegible]L[illegible]AEE[illegible]RLYLE[illegible]T[illegible]YKT
(457) V[illegible]RSAT[illegible]LIR[illegible]L[illegible]L[illegible]HAEE[illegible]REYLE[illegible]T[illegible]C[illegible]YKT
(456) V[illegible]LKSAT[illegible]ITR[illegible]L[illegible]AEE[illegible]REYLE[illegible]YKT
(460) VDYVPLKSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKT
                                                            Section 11
(511) 511          524
(509) L[illegible]SFSA[illegible]          SEQ ID NO: 10073
(508) FDF[illegible]L[illegible]NTFTKL[illegible]     SEQ ID NO: 10074
(508) FDF[illegible]NL[illegible]NTFTRL[illegible]    SEQ ID NO: 10075
(507) FDF[illegible]WNTFTK[illegible]      SEQ ID NO: 10076
(511) FDFYNLWNTFTKLQ     SEQ ID NO: 10077
```

FIGURE 119

```
tagtcaaaacccacagaatgattccagcaggcataagtatctgatgaagtagaaaagcaa
 -                   -                   -  V  S  D  E  V  E  K  Q
gttgcacgtTTGtcacacagacaacacgttctttcaggtccaatcTTGacaaagtacttc
 V  A  R  L  S  H  R  Q  H  V  L  S  G  P  I  L  T  K  Y  F
attgatgtaagctcaaagccatgcgcccaaaggacgaacacgactctgtctgacaatcct
 I  D  V  S  S  K  P  C  A  Q  R  T  N  T  T  L  S  D  N  P
ttcagtgtatcactgagcatttgtactatcttaatacgcactacattccagggcaagcct
 F  S  V  S  L  S  I  C  T  I  L  I  R  T  T  F  Q  G  K  P
ttatacATGagtggtataagatgtttaaactgctcactggtggaggtttgtgcattaact
 L  Y  M  S  G  I  R  C  L  N  C  S  L  V  E  V  C  A  L  T
Ctggtgaatttgtgttattttcagtgtcaacataa SEQ ID NO: 10080
 L  V  N  F  V  L  F  S  V  S  T  -  SEQ ID NO: 10027
```

FIGURE 120

FIGURE 120A

PRHTQRT-PTVDSSL-WVSK-ITKSMVTLICLSPAKKLFVTFVRGLALM-RAVMQ
LEMLWVLTYLSS-DFLQVLT--LYRLVMLTLKITQNSPELMQNLHQVTSLNILYH
SCIKACPGM-CVLR-YKCSVIH-KDCQTESCSSFGRMALSLHQ-STLSRLDLKER
VVCVTNVQLAFLLHQILMPAGIILWVLTMSITHL-LMFSSGALRVTFRVTMTNIA
RYMEMHMWLVVMLS-LDV-QSMSALLSALIGLLNTLL-EMN-GLILLAEKYNTWL
-SLHCLLISFQFFMT-EIQRLSSVCLRLK-NGSSTMLSHVVTKLTK-RNSSILML
YITINSLMVFVCFGIVTLIVTQPMQLCVGLTQESCQT-TYQAVMVVVCM-ISMHS
TLQLSIKVHLLI-SNCLSFTILIVLVSLMANK-CRILIMFHSNLLRVLHDAI-VV
LFADTMQMSTDSTWMHII--FLLDLAYGFTNNLILITCGIHLPGYRV

FIGURE 120B

LGIPKGHDLP-THLYDGFQNELPSQWLP-YVYHPRRSYSSRSCVDWL-CRGLSCN
-RCCGY-PTSPARIFYRC-LSSCTDWLC-H-K-HRIHQS-CKTSTR-PV-TSYTT
HV-RLALECSAY-DSTNAQ-YTERIVRQSRVRPLGAWL-AYINEVLCQDWT-KNV
LSV-QTCNLLFYFIRYLCLLESFCGF-LCL-PIYD-CSAVGLYG-PSE-P-PTLP
GTWKCTCG-L-CYHD-MFSSP-VLC-AR-LVC-IPYYRR-TEG-FCLQKSTTHGC
EVCIAC--VSSSS-HRKSKGYQVCASG-SRMEVLRCSAM--QSLQNRGTLLFLCY
TSR-IH-WCLFVLEL-R-SLPSQCNCV-V-HKSLVKLELTRL-WW-FVCE-ACIP
HSSFR-KCIY-FKAIAFLLLF--SL-VSWQTSSVGY-LCSTQICYVYYTMQFRWC
CLQTPCK-VPTVLGCI-YDDFCWI-PMDLQTI-YL-PVEYIYQVTEF

FIGURE 120C

-AYPKDMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVG
TNLPLQLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVV
RIKIVQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSD
TYACWNHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAV
HECFVKRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVP
QAEVEWKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFD
TRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSD
IDYVPLKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNT
FTRLQSL

FIGURE 120D

-TL-PGKCIPQVISIKLFVNP-AKSSRNHHIICIQVLSVLICMVSANSTT-IASCNTRSR
FEWNIINIRHYLFAMRLTRTIRIVKERQLL-ISKCTFIESWSVECMLIHIQTTTITAW-V
QV-QDSCVKPTHNCIGWVTINVTIPKQTNTISEFIVMYSIRIEEFLYFVSFVTTWLSIVE
LPFYFSLRHTLDSLWISYVMKNWKLISKQCRLHNHVLYFSASRINPQFISYNRVFNRPIN
ALNKALMDC-TSSHDSITTSHMCISMYLAMLVMVTLKVTRKAPLLNINHKWVIDIVKTHR
MIPAGISI--SRKASCTFVTQTTRSFRSNLDKVLH-CKLKAMRPKDEHDSV-QSFQCITE
HLYYLNTHYIPGQAFIHEWYKMFKLVTWWRFCINSGEFCVIFSVNITSRYSY-VNTCRKS
-LER-VSTHSISSCMTALYIKANPRTNVTNSFFAGDKHIRVTIDLVIHFETHHRDESTVG
HVLWVCL

FIGURE 120E

KLCNLVNVFHRL-VSNCL-IHRLNPAEIIIILYASKYCRYSFAWCLQTAPPKLHRVIHVAD
LSGT-SISDTTCLP-DSQGLSE--KKGNCFKLVNALLSKAGVWNACLFTYKLPPSQPGKF
KFDKTLVSNLHTIALAG-RSTLQFQNKQTPSVNLS-CIA-E-KSSSIL-ALSLHG-AS-N
FHSTSA-GTHLIAFGFPMS-RTGNLSASNADFTTMCCTFLQAELTLSSSPIIGYSTDQST
RLTKHSWTAKHLVMIASQLATCAFPCTWQCWSWLL-RLPVKPHC-TSIINGL-T-SKPTE
-FQQA-VSDEVEKQVARLSHRQHVLSGPILTKYFIDVSSKPCAQRTNTTLSDNPFSVSLS
ICTILIRTTFQGKPLYMSGIRCLNWSPGGGFALTLVNSVLFSVST-PVGTATKLTPVENP
SWRGRLVPTASLVA-QPSTSKPIHART-RIASSRVINILG-PLTW-FILKPIIEMSLR-V
MSFGYA-

FIGURE 120F

NSVTW-MYSTGYKYQIVCKSIG-IQQKSSYYMHPSTVGTHLHGVCKQHHLNCIV-YT-QI
-VEHNQYPTLLVCHETHKDYQNSKRKAIALN--MHFYRKLECGMHAYSHTNYHHHSLVSS
SLTRLLCQTYTQLHWLGNDQRYNSKTNKHHQ-IYRDV-HKNRRVPLFCKLCHYMAEHRRT
SILLQPEAHT--PLDFLCHEELETYQQAMQTSQPCVVLFCKQN-PSVHLL--GIQQTNQR
A-QSTHGLLNI-S--HHN-PHVHFHVPGNVGHGYSEGYP-SPTAEHQS-MGYRHSQNPQN
DSSRHKYLMK-KSKLHVCHTDNTFFQVQS-QSTSLM-AQSHAPKGRTRLCLTILSVYH-A
FVLS-YALHSRASLYT-VV-DV-TGHLVEVLH-LW-ILCYFQCQHNQSVQLLS-HL-KIL
AGEVG-YPQHL-LHDSPLHQSQSTHERDE-LLRG--TY-GNH-LGNSF-NPS-R-VYGRS
CPLGMPR

FIGURE 121

```
                         10        20        30        40        50        60
                          |         |         |         |         |         |
SEQ ID NO:10033 --------------------------TACCGTAGACTCATCTCTATGATGGGTTTCAAAA
SEQ ID NO:10084 CCTAGGCATACCCAAAGGACATGACCTACCGTAGACTCATCTCTATGATGGGTTTCAAAA
Consensus                                 TACCGTAGACTCATCTCTATGATGGGTTTCAAAA
Prim. cons.     CCTAGGCATACCCAAAGGACATGACCTACCGTAGACTCATCTCTATGATGGGTTTCAAAA

                         70        80        90       100       110       120
                          |         |         |         |         |         |
SEQ ID NO:10033 TGAATTACCAAGTCAATGGTTACCCTAATATGTTTATCACCCGCGAAGAAGCTATTCGTC
SEQ ID NO:10084 TGAATTACCAAGTCAATGGTTACCCTAATATGTTTATCACCCGCGAAGAAGCTATTCGTC
Consensus       TGAATTACCAAGTCAATGGTTACCCTAATATGTTTATCACCCGCGAAGAAGCTATTCGTC
Prim. cons.     TGAATTACCAAGTCAATGGTTACCCTAATATGTTTATCACCCGCGAAGAAGCTATTCGTC

                        130       140       150       160       170       180
                          |         |         |         |         |         |
SEQ ID NO:10033 ACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCATGCAACTAGAGATGCTGTGG
SEQ ID NO:10084 ACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCATGCAACTAGAGATGCTGTGG
Consensus       ACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCATGCAACTAGAGATGCTGTGG
Prim. cons.     ACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCATGCAACTAGAGATGCTGTGG

                        190       200       210       220       230       240
                          |         |         |         |         |         |
SEQ ID NO:10033 GTACTAACCTACCTCTCCAGCTAGGATTTTCTACAGGTGTTAACTTAGTAGCTGTACCGA
SEQ ID NO:10084 GTACTAACCTACCTCTCCAGCTAGGATTTTCTACAGGTGTTAACTTAGTAGCTGTACCGA
Consensus       GTACTAACCTACCTCTCCAGCTAGGATTTTCTACAGGTGTTAACTTAGTAGCTGTACCGA
Prim. cons.     GTACTAACCTACCTCTCCAGCTAGGATTTTCTACAGGTGTTAACTTAGTAGCTGTACCGA

                        250       260       270       280       290       300
                          |         |         |         |         |         |
SEQ ID NO:10033 CTGGTTATGTTGACACTGAAAATAACACAGAATTCACCAGAGTTAATGCAAAACCTCCAC
SEQ ID NO:10084 CTGGTTATGTTGACACTGAAAATAACACAGAATTCACCAGAGTTAATGCAAAACCTCCAC
Consensus       CTGGTTATGTTGACACTGAAAATAACACAGAATTCACCAGAGTTAATGCAAAACCTCCAC
Prim. cons.     CTGGTTATGTTGACACTGAAAATAACACAGAATTCACCAGAGTTAATGCAAAACCTCCAC

                        310       320       330       340       350       360
                          |         |         |         |         |         |
SEQ ID NO:10033 CAGGTGACCAGTTTAAACATCTTATACC--------------------------------
SEQ ID NO:10084 CAGGTGACCAGTTTAAACATCTTATACCACTCATGTATAAAGGCTTGCCCTGGAATGTAG
Consensus       CAGGTGACCAGTTTAAACATCTTATACC
Prim. cons.     CAGGTGACCAGTTTAAACATCTTATACCACTCATGTATAAAGGCTTGCCCTGGAATGTAG
```

etc.

FIGURE 122

5'3' Frame 1

```
cctaggcatacccaaaggacatgacctaccgtagactcatctctatgatgggtttcaaaa
 P  R  H  T  Q  R  T  -  P  T  V  D  S  S  L  -  W  V  S  K
tgaattaccaagtcaatggttaccctaatatgtttatcacccgcgaagaagctattcgtc
 -  I  T  K  S  M  V  T  L  I  C  L  S  P  A  K  K  L  F  V
acgttcgtgcgtggattggctttgatgtagagggctgtcatgcaactagagatgctgtgg
 T  F  V  R  G  L  A  L  M  -  R  A  V  M  Q  L  E  M  L  W
gtactaacctacctctccagctaggatttctacaggtgttaacttagtagctgtaccga
 V  L  T  Y  L  S  S  -  D  F  L  Q  V  L  T  -  -  L  Y  R
ctggttatgttgacactgaaaataacacagaattcaccagagttaatgcaaaacctccac
 L  V  M  L  T  L  K  I  T  Q  N  S  P  E  L  M  Q  N  L  H
caggtgaccagtttaaacatcttataccactcatgtataaaggcttgccctggaatgtag
 Q  V  T  S  L  N  I  L  Y  H  S  C  I  K  A  C  P  G  M  -
tgcgtattaagatagtacaaatgctcagtgatacactgaaaggattgtcagacagagtcg
 C  V  L  R  -  Y  K  C  S  V  I  H  -  K  D  C  Q  T  E  S
tgttcgtcctttgggcgcatggctttgagcttacatcaatgaagtactttgtcaagattg
 C  S  S  F  G  R  M  A  L  S  L  H  Q  -  S  T  L  S  R  L
gacctgaaagaacgtgttgtctgtgtgacaaacgtgcaacttgctttctacttcatcag
 D  L  K  E  R  V  V  C  V  T  N  V  Q  L  A  F  L  L  H  Q
atacttatgcctgctggaatcattctgtgggtttgactatgtctataacccatttatga
 I  L  M  P  A  G  I  I  L  W  V  L  T  M  S  I  T  H  L  -
ttgatgttcagcagtggggctttacgggtaaccttcagagtaaccatgaccaacattgcc
 L  M  F  S  S  G  A  L  R  V  T  F  R  V  T  M  T  N  I  A
aggtacatggaaatgcacatgtggctagttgtgatgctatcatgactagatgtttagcag
 R  Y  M  E  M  H  M  W  L  V  V  M  L  S  -  L  D  V  -  Q
tccatgagtgctttgttaagcgcgttgattggtctgttgaataccctattataggagatg
 S  M  S  A  L  L  S  A  L  I  G  L  L  N  T  L  L  -  E  M
aactgagggttaattctgcttgcagaaaagtacaacacatggttgtgaagtctgcattgc
 N  -  G  L  I  L  L  A  E  K  Y  N  T  W  L  -  S  L  H  C
ttgctgataagtttccagttcttcatgacataggaaatccaaaggctatcaagtgtgtgc
 L  L  I  S  F  Q  F  F  M  T  -  E  I  Q  R  L  S  S  V  C
ctcaggctgaagtagaatggaagttctacgatgctcagccatgtagtgacaaagcttaca
 L  R  L  K  -  N  G  S  S  T  M  L  S  H  V  V  T  K  L  T
aaatagaggaactcttctattcttatgctatacatcacgataaattcactgatggtgttt
 K  -  R  N  S  S  I  L  M  L  Y  I  T  I  N  S  L  M  V  F
gtttgttttggaattgtaacgttgatcgttacccagccaatgcaattgtgtgtaggtttg
 V  C  F  G  I  V  T  L  I  V  T  Q  P  M  Q  L  C  V  G  L
acacaagagtcttgtcaaacttgaacttaccaggctgtgatggtggtagtttgtatgtga
 T  Q  E  S  C  Q  T  -  T  Y  Q  A  V  M  V  V  V  C  M  -
ataagcatgcattccacactccagctttcgataaaagtgcatttactaatttaaagcaat
 I  S  M  H  S  T  L  Q  L  S  I  K  V  H  L  L  I  -  S  N
tgcctttcttttactattctgatagtccttgtgagtctcatggcaaacaagtagtgtcgg
 C  L  S  F  T  I  L  I  V  L  V  S  L  M  A  N  K  -  C  R
atattgattatgttccactcaaatctgctacgtgtattacacgatgcaatttaggtggtg
 I  L  I  M  F  H  S  N  L  L  R  V  L  H  D  A  I  -  V  V
ctgtttgcagacaccatgcaaatgagtaccgacagtacttggatgcatataatatgatga
 L  F  A  D  T  M  Q  M  S  T  D  S  T  W  M  H  I  I  -  -
tttctgctggatttagcctatggatttacaaacaatttgatacttataacctgtggaata
 F  L  L  D  L  A  Y  G  F  T  N  N  L  I  L  I  T  C  G  I
```

catttaccaggttacagagttta
 H L P G Y R V

5'3' Frame 2

cctaggcatacccaaaggacatgacctaccgtagactcatctctatgatgggtttcaaaat
 L G I P K G H D L P - T H L Y D G F Q N
gaattaccaagtcaatggttaccctaatatgtttatcacccgcgaagaagctattcgtca
 E L P S Q W L P - Y V Y H P R R S Y S S
cgttcgtgcgtggattggctttgatgtagagggctgtcatgcaactagagatgctgtggg
 R S C V D W L - C R G L S C N - R C C G
tactaacctacctctccagctaggattttctacaggtgttaacttagtagctgtaccgac
 Y - P T S P A R I F Y R C - L S S C T D
tggttatgttgacactgaaaataacacagaattcaccagagttaatgcaaaacctccacc
 W L C - H - K - H R I H Q S - C K T S T
aggtgaccagtttaaacatcttataccactcatgtataaaggcttgccctggaatgtagt
 R - P V - T S Y T T H V - R L A L E C S
gcgtattaagatagtacaaatgctcagtgatacactgaaaggattgtcagacagagtcgt
 A Y - D S T N A Q - Y T E R I V R Q S R
gttcgtcctttgggcgcatggctttgagcttacatcaatgaagtactttgtcaagattgg
 V R P L G A W L - A Y I N E V L C Q D W
acctgaaagaacgtgttgtctgtgtgacaaacgtgcaacttgctttctacttcatcaga
 T - K N V L S V - Q T C N L L F Y F I R
tacttatgcctgctggaatcattctgtgggttttgactatgtctataacccatttatgat
 Y L C L L E S F C G F - L C L - P I Y D
tgatgttcagcagtggggctttacgggtaaccttcagagtaaccatgaccaacattgcca
 - C S A V G L Y G - P S E - P - P T L P
ggtacatggaaatgcacatgtggctagttgtgatgctatcatgactagatgtttagcagt
 G T W K C T C G - L - C Y H D - M F S S
ccatgagtgctttgttaagcgcgttgattggtctgttgaataccctattataggagatga
 P - V L C - A R - L V C - I P Y Y R R -
actgagggttaattctgcttgcagaaaagtacaacacatggttgtgaagtctgcattgct
 T E G - F C L Q K S T T H G C E V C I A
tgctgataagtttccagttcttcatgacataggaaatccaaaggctatcaagtgtgtgcc
 C - - V S S S S - H R K S K G Y Q V C A
tcaggctgaagtagaatggaagttctacgatgctcagccatgtagtgacaaagcttacaa
 S G - S R M E V L R C S A M - - Q S L Q
aatagaggaactcttctattcttatgctatacatcacgataaattcactgatggtgtttg
 N R G T L L F L C Y T S R - I H - W C L
tttgttttggaattgtaacgttgatcgttacccagccaatgcaattgtgtgtaggtttga
 F V L E L - R - S L P S Q C N C V - V -
cacaagagtcttgtcaaacttgaacttaccaggctgtgatggtggtagtttgtatgtgaa
 H K S L V K L E L T R L - W W - F V C E
taagcatgcattccacactccagctttcgataaaagtgcatttactaatttaaagcaatt
 - A C I P H S S F R - K C I Y - F K A I
gcctttcttttactattctgatagtccttgtgagtctcatggcaacaagtagtgtcgga
 A F L L L F - - S L - V S W Q T S S V G
tattgattatgttccactcaaatctgctacgtgtattacacgatgcaatttaggtggtgc
 Y - L C S T Q I C Y V Y Y T M Q F R W C
tgtttgcagacaccatgcaaatgagtaccgacagtacttggatgcatataatatgatgat

C L Q T P C K - V P T V L G C I - Y D D
ttctgctggatttagcctatggatttacaaacaatttgatacttataacctgtggaatac
F C W I - P M D L Q T I - Y L - P V E Y
atttaccaggttacagagttta
I Y Q V T E F

5'3' Frame 3

cctaggcatacccaaaggacATGacctaccgtagactcatctctatgatgggtttcaaatg
- A Y P K D M T Y R R L I S M M G F K M
aattaccaagtcaatggttaccctaatatgtttatcacccgcgaagaagctattcgtcac
N Y Q V N G Y P N M F I T R E E A I R H
gttcgtgcgtggattggctttgatgtagagggctgtcatgcaactagagatgctgtgggt
V R A W I G F D V E G C H A T R D A V G
actaacctacctctccagctaggatttctacaggtgttaacttagtagctgtaccgact
T N L P L Q L G F S T G V N L V A V P T
ggttatgttgacactgaaaataacacagaattcaccagagttaatgcaaaacctccacca
G Y V D T E N N T E F T R V N A K P P P
ggtgaccagtttaaacatcttataccactcatgtataaaggcttgccctggaatgtagtg
G D Q F K H L I P L M Y K G L P W N V V
cgtattaagatagtacaaatgctcagtgatacactgaaaggattgtcagacagagtcgtg
R I K I V Q M L S D T L K G L S D R V V
ttcgtcctttgggcgcatggctttgagcttacatcaatgaagtactttgtcaagattgga
F V L W A H G F E L T S M K Y F V K I G
cctgaaagaacgtgttgtctgtgtgacaaacgtgcaacttgcttttctacttcatcagat
P E R T C C L C D K R A T C F S T S S D
acttatgcctgctggaatcattctgtgggttttgactatgtctataacccatttatgatt
T Y A C W N H S V G F D Y V Y N P F M I
gatgttcagcagtggggctttacgggtaaccttcagagtaaccatgaccaacattgccag
D V Q Q W G F T G N L Q S N H D Q H C Q
gtacatggaaatgcacatgtggctagttgtgatgctatcatgactagatgtttagcagtc
V H G N A H V A S C D A I M T R C L A V
catgagtgctttgttaagcgcgttgattggtctgttgaatacccctattataggagatgaa
H E C F V K R V D W S V E Y P I I G D E
ctgagggttaattctgcttgcagaaaagtacaacacatggttgtgaagtctgcattgctt
L R V N S A C R K V Q H M V V K S A L L
gctgataagtttccagttcttcatgacataggaaatccaaaggctatcaagtgtgtgcct
A D K F P V L H D I G N P K A I K C V P
caggctgaagtagaatggaagttctacgatgctcagccatgtagtgacaaagcttacaaa
Q A E V E W K F Y D A Q P C S D K A Y K
atagaggaactcttctattcttatgctatacatcacgataaattcactgatggtgtttgt
I E E L F Y S Y A I H H D K F T D G V C
ttgttttggaattgtaacgttgatcgttacccagccaatgcaattgtgtgtaggtttgac
L F W N C N V D R Y P A N A I V C R F D
acaagagtcttgtcaaacttgaacttaccaggctgtgatggtggtagtttgtatgtgaat
T R V L S N L N L P G C D G G S L Y V N
aagcatgcattccacactccagctttcgataaaagtgcatttactaatttaaagcaattg
K H A F H T P A F D K S A F T N L K Q L
cctttcttttactattctgatagtccttgtgagtctcatggcaaacaagtagtgtcggat
P F F Y Y S D S P C E S H G K Q V V S D

attgattatgttccactcaaatctgctacgtgtattacacgatgcaatttaggtggtgct
I D Y V P L K S A T C I T R C N L G G A
gtttgcagacaccatgcaaatgagtaccgacagtacttggatgcatataatatgatgatt
V C R H H A N E Y R Q Y L D A Y N M M I
tctgctggatttagcctatggatttacaaacaatttgatacttataacctgtggaataca
S A G F S L W I Y K Q F D T Y N L W N T
tttaccaggttacagagttta
F T R L Q S L

3'5' Frame 1

taaactctgtaacctggtaaatgtattccacaggttataagtatcaaattgtttgtaaat
- T L - P G K C I P Q V I S I K L F V N
ccataggctaaatccagcagaaatcatcatattatatgcatccaagtactgtcggtactc
P - A K S S R N H H I I C I Q V L S V L
atttgcatggtgtctgcaaacagcaccacctaaattgcatcgtgtaatacacgtagcaga
I C M V S A N S T T - I A S C N T R S R
tttgagtggaacataatcaatatccgacactacttgtttgccatgagactcacaaggact
F E W N I I N I R H Y L F A M R L T R T
atcagaatagtaaaagaaaggcaattgctttaaattagtaaatgcacttttatcgaaagc
I R I V K E R Q L L - I S K C T F I E S
tggagtgtggaatgcatgcttattcacatacaaactaccaccatcacagcctggtaagtt
W S V E C M L I H I Q T T T I T A W - V
caagtttgacaagactcttgtgtcaaacctacacacaattgcattggctgggtaacgatc
Q V - Q D S C V K P T H N C I G W V T I
aacgttacaattccaaaacaaacaaacaccatcagtgaatttatcgtgatgtatagcata
N V T I P K Q T N T I S E F I V M Y S I
agaatagaagagttcctctattttgtaagctttgtcactacatggctgagcatcgtagaa
R I E E F L Y F V S F V T T W L S I V E
cttccattctacttcagcctgaggcacacacttgatagcctttggatttcctatgtcatg
L P F Y F S L R H T L D S L W I S Y V M
aagaactggaaacttatcagcaagcaatgcagacttcacaaccatgtgttgtacttttct
K N W K L I S K Q C R L H N H V L Y F S
gcaagcagaattaaccctcagttcatctcctataatagggtattcaacagaccaatcaac
A S R I N P Q F I S Y N R V F N R P I N
gcgcttaacaaagcactcatggactgctaaacatctagtcatgatagcatcacaactagc
A L N K A L M D C - T S S H D S I T T S
cacatgtgcatttccatgtacctggcaatgttggtcatggttactctgaaggttacccgt
H M C I S M Y L A M L V M V T L K V T R
aaagccccactgctgaacatcaatcataaatgggttatagacatagtcaaaacccacaga
K A P L L N I N H K W V I D I V K T H R
atgattccagcaggcataagtatctgatgaagtagaaaagcaagttgcacgtttgtcaca
M I P A G I S I - - S R K A S C T F V T
cagacaacacgttctttcaggtccaatcttgacaaagtacttcattgatgtaagctcaaa
Q T T R S F R S N L D K V L H - C K L K
gccatgcgcccaaaggacgaacacgactctgtctgacaatcctttcagtgtatcactgag
A M R P K D E H D S V - Q S F Q C I T E
catttgtactatcttaatacgcactacattccagggcaagcctttatacatgagtggtat
H L Y Y L N T H Y I P G Q A F I H E W Y
aagatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattctgtgtt

K M F K L V T W W R F C I N S G E F C V
attttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaaaatcc
I F S V N I T S R Y S Y - V N T C R K S
tagctggagaggtaggttagtacccacagcatctctagttgcatgacagccctctacatc
- L E R - V S T H S I S S C M T A L Y I
aaagccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacatattagg
K A N P R T N V T N S F F A G D K H I R
gtaaccattgacttggtaattcatttgaaacccatcatagagatgagtctacggtaggt
V T I D L V I H F E T H H R D E S T V G
catgtcctttgggtatgcctagg
H V L W V C L

3'5' Frame 2

taaactctgtaacctggtaaatgtattccacaggttataagtatcaattgtttgtaaatc
K L C N L V N V F H R L - V S N C L - I
cataggctaaatccagcagaaatcatcatattatatgcatccaagtactgtcggtactca
H R L N P A E I I I L Y A S K Y C R Y S
tttgcatggtgtctgcaaacagcaccacctaaattgcatcgtgtaatacacgtagcagat
F A W C L Q T A P P K L H R V I H V A D
ttgagtggaacataatcaatatccgacactacttgtttgccatgagactcacaaggacta
L S G T - S I S D T T C L P - D S Q G L
tcagaatagtaaaagaaaggcaattgctttaaattagtaaatgcacttttatcgaaagct
S E - - K K G N C F K L V N A L L S K A
ggagtgtggaatgcatgcttattcacatacaaactaccaccatcacagcctggtaagttc
G V W N A C L F T Y K L P P S Q P G K F
aagtttgacaagactcttgtgtcaaacctacacacaattgcattggctgggtaacgatca
K F D K T L V S N L H T I A L A G - R S
acgttacaattccaaaacaaacaaacaccatcagtgaatttatcgtgatgtatagcataa
T L Q F Q N K Q T P S V N L S - C I A -
gaatagaagagttcctctatttgtaagctttgtcactacatggctgagcatcgtagaac
E - K S S S I L - A L S L H G - A S - N
ttccattctacttcagcctgaggcacacacttgatagcctttggatttcctatgtcatga
F H S T S A - G T H L I A F G F P M S -
agaactggaaacttatcagcaagcaatgcagacttcacaaccatgtgttgtacttttctg
R T G N L S A S N A D F T T M C C T F L
caagcagaattaaccctcagttcatctcctataatagggtattcaacagaccaatcaacg
Q A E L T L S S S P I I G Y S T D Q S T
cgcttaacaaagcactcatggactgctaaacatctagtcatgatagcatcacaactagcc
R L T K H S W T A K H L V M I A S Q L A
acatgtgcatttccatgtacctggcaatgttggtcatggttactctgaaggttacccgta
T C A F P C T W Q C W S W L L - R L P V
aagccccactgctgaacatcaatcataaatgggttatagacatagtcaaaacccacagaa
K P H C - T S I I N G L - T - S K P T E
tgattccagcaggcataagtatctgatgaagtagaaaagcaagttgcacgtttgtcacac
- F Q Q A - V S D E V E K Q V A R L S H
agacaacacgttctttcaggtccaatcttgacaaagtacttcattgatgtaagctcaaag
R Q H V L S G P I L T K Y F I D V S S K
ccatgcgcccaaaggacgaacacgactctgtctgacaatcctttcagtgtatcactgagc
P C A Q R T N T T L S D N P F S V S L S

atttgtactatcttaatacgcactacattccagggcaagcctttatacatgagtggtata
 I C T I L I R T T F Q G K P L Y M S G I
agatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattctgtgtta
 R C L N W S P G G G F A L T L V N S V L
ttttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaaaatcct
 F S V S T - P V G T A T K L T P V E N P
agctggagaggtaggttagtacccacagcatctctagttgcatgacagccctctacatca
 S W R G R L V P T A S L V A - Q P S T S
aagccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacatattaggg
 K P I H A R T - R I A S S R V I N I L G
taaccattgacttggtaattcatttgaaacccatcatagagatgagtctacggtaggtc
 - P L T W - F I L K P I I E M S L R - V
atgtcctttgggtatgcctagg
 M S F G Y A -

3'5' Frame 3

taaactctgtaacctggtaaatgtattccacaggttataagtatcaaattgtttgtaaatcc
   N S V T W - M Y S T G Y K Y Q I V C K S
ataggctaaatccagcagaaatcatcatattatatgcatccaagtactgtcggtactcat
 I G - I Q Q K S S Y Y M H P S T V G T H
ttgcatggtgtctgcaaacagcaccacctaaattgcatcgtgtaatacacgtagcagatt
 L H G V C K Q H H L N C I V - Y T - Q I
tgagtggaacataatcaatatccgacactacttgtttgccatgagactcacaaggactat
 - V E H N Q Y P T L L V C H E T H K D Y
cagaatagtaaaagaaaggcaattgctttaaattagtaaatgcacttttatcgaaagctg
 Q N S K R K A I A L N - - M H F Y R K L
gagtgtggaatgcatgcttattcacatacaaactaccaccatcacagcctggtaagttca
 E C G M H A Y S H T N Y H H H S L V S S
agtttgacaagactcttgtgtcaaacctacacacaattgcattggctgggtaacgatcaa
 S L T R L L C Q T Y T Q L H W L G N D Q
cgttacaattccaaaacaaacaaacaccatcagtgaatttatcgtgatgtatagcataag
 R Y N S K T N K H H Q - I Y R D V - H K
aatagaagagttcctctatttgtaagctttgtcactacatggctgagcatcgtagaact
 N R R V P L F C K L C H Y M A E H R R T
tccattctacttcagcctgaggcacacacttgatagcctttggatttcctatgtcatgaa
 S I L L Q P E A H T - - P L D F L C H E
gaactggaaacttatcagcaagcaatgcagacttcacaaccatgtgttgtactttctgc
 E L E T Y Q Q A M Q T S Q P C V V L F C
aagcagaattaaccctcagttcatctcctataatagggtattcaacagaccaatcaacgc
 K Q N - P S V H L L - - G I Q Q T N Q R
gcttaacaaagcactcatggactgctaaacatctagtcatgatagcatcacaactagcca
 A - Q S T H G L L N I - S - - H H N - P
catgtgcatttccatgtacctggcaatgttggtcatggttactctgaaggttacccgtaa
 H V H F H V P G N V G H G Y S E G Y P -
agccccactgctgaacatcaatcataaatgggttatagacatagtcaaaacccacagaat
 S P T A E H Q S - M G Y R H S Q N P Q N
gattccagcaggcataagtatctgatgaagtagaaaagcaagttgcacgtttgtcacaca
 D S S R H K Y L M K - K S K L H V C H T
gacaacacgttctttcaggtccaatcttgacaaagtacttcattgatgtaagctcaaagc

D N T F F Q V Q S - Q S T S L M - A Q S
catgcgcccaaaggacgaacacgactctgtctgacaatcctttcagtgtatcactgagca
H A P K G R T R L C L T I L S V Y H - A
tttgtactatcttaatacgcactacattccagggcaagcctttatacatgagtggtataa
F V L S - Y A L H S R A S L Y T - V V -
gatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattctgtgttat
D V - T G H L V E V L H - L W - I L C Y
tttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaaaatccta
F Q C Q H N Q S V Q L L S - H L - K I L
gctggagaggtaggttagtacccacagcatctctagttgcatgacagccctctacatcaa
A G E V G - Y P Q H L - L H D S P L H Q
agccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacatattagggt
S Q S T H E R D E - L L R G - - T Y - G
aaccattgacttggtaattcatttgaaacccatcatagagatgagtctacggtaggtca
N H - L G N S F - N P S - R - V Y G R S
tgtcctttgggtatgcctagg
C P L G M P R

FIGURE 123

CCTAGGCATACCCAAAGGACATGACCTACCGTAGACTCATCTCTATGATGGGTTTCAAAATGAATTACCAAGTCAATGGT
...................i.....................N..N.........i..............N....
TACCCTAATATGTTTATCACCCGCGAAGAAGCTATTCGTCACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCA
.........i...N.............N
TGCAACTAGAGATGCTGTGGGTACTAACCTACCTCTCCAGCTAGGATTTTCTACAGGTGTTAACTTAGTAGCTGTACCGA
...........N...
CTGGTTATGTTGACACTGAAAATAACACAGAATTCACCAGAGTTAATGCAAAACCTCCACCAGGTGACCAGTTTAAACAT
......N...................................N....................................
CTTATACCACTCATGTATAAAGGCTTGCCCTGGAATGTAGTGCGTATTAAGATAGTACAAATGCTCAGTGATACACTGAA
...........N....................N.......................N..................
AGGATTGTCAGACAGAGTCGTGTTCGTCCTTTGGGCGCATGGCTTTGAGCTTACATCAATGAAGTACTTTGTCAAGATTG
..................................N.................N....................
GACCTGAAAGAACGTGTTGTCTGTGTGACAAACGTGCAACTTGCTTTTCTACTTCATCAGATACTTATGCCTGCTGGAAT
....N............
CATTCTGTGGGTTTTGACTATGTCTATAACCCATTTATGATTGATGTTCAGCAGTGGGGCTTTACGGGTAACCTTCAGAG
...................N...............N.....N...................................
TAACCATGACCAACATTGCCAGGTACATGGAAATGCACATGTGGCTAGTTGTGATGCTATCATGACTAGATGTTTAGCAG
.....N..................N....N....N.............N......i.......N.........
TCCATGAGTGCTTTGTTAAGCGCGTTGATTGGTCTGTTGAATACCCTATTATAGGAGATGAACTGAGGGTTAATTCTGCT
...N..N..................
TGCAGAAAAGTACAACACATGGTTGTGAAGTCTGCATTGCTTGCTGATAAGTTTCCAGTTCTTCATGACATAGGAAATCC
..................i...N..........
AAAGGCTATCAAGTGTGTGCCTCAGGCTGAAGTAGAATGGAAGTTCTACGATGCTCAGCCATGTAGTGACAAAGCTTACA
...................................N.............N........N..................
AAATAGAGGAACTCTTCTATTCTTATGCTATACATCACGATAAATTCACTGATGGTGTTTGTTTGTTTTGGAATTGTAAC
.......................N.........................N.............................
GTTGATCGTTACCCAGCCAATGCAATTGTGTGTAGGTTTGACACAAGAGTCTTGTCAAACTTGAACTTACCAGGCTGTGA
...................N...N
TGGTGGTAGTTTGTATGTGAATAAGCATGCATTCCACACTCCAGCTTTCGATAAAAGTGCATTTACTAATTTAAAGCAAT
..............N..........N...
TGCCTTTCTTTTACTATTCTGATAGTCCTTGTGAGTCTCATGGCAAACAAGTAGTGTCGGATATTGATTATGTTCCACTC
....i...........................N..........
AAATCTGCTACGTGTATTACACGATGCAATTTAGGTGGTGCTGTTTGCAGACACCATGCAAATGAGTACCGACAGTACTT
......................N..............................N.....N..................
GGATGCATATAATATGATGATTTCTGCTGGATTTAGCCTATGGATTTACAAACAATTTGATACTTATAACCTGTGGAATA
..N.........N..N.....................N...
CATTTACCAGGTTACAGAGTTTA **SEQ ID NO: 10084**
....

FIGURE 123 (contd.)

Pos	Score	Pred
21	0.651	Yes
45	0.354	–
48	0.387	–
60	0.590	Yes
76	0.470	–
90	0.676	Yes
145	0.192	–
160	0.410	–
172	0.290	–
247	0.221	–
286	0.219	–
333	0.373	–
355	0.178	–
381	0.286	–
439	0.405	–
459	0.204	–
547	0.289	–
580	0.447	–
597	0.449	–
604	0.290	–
646	0.427	–
667	0.427	–
673	0.208	–
679	0.317	–
694	0.180	–
702	0.554	Yes
710	0.151	–
724	0.384	–
778	0.151	–
819	0.711	Yes
865	0.306	–
917	0.230	–
931	0.214	–
941	0.190	–
985	0.274	–
1012	0.368	–
1060	0.206	–
1120	0.193	–
1135	0.185	–
1147	0.431	–
1240	0.562	Yes
1270	0.377	–
1304	0.190	–
1336	0.353	–
1342	0.312	–
1363	0.213	–
1374	0.178	–
1377	0.096	–
1400	0.056	–

FIGURE 124

```
Sequences:                                                          (bits) Value

gi|74827|pir||VFIHJH  genome polyprotein 1b - murine hepatit...   638    0.0
gi|14917044|sp|P29982|RRPB_CVMJH  RNA-directed RNA polymeras...   637    0.0
gi|26007546|ref|NP_068668.2|  ORF1ab polyprotein [Murine hep...   637    0.0
gi|7769342|gb|AAF69332.1|AF208066_2  RNA-directed RNA polyme...   637    0.0
gi|6625761|gb|AAF19384.1|AF201929_2  RNA-directed RNA polyme...   637    0.0
gi|2641128|gb|AAB86818.1|  RNA-directed RNA polymerase [muri...   635    0.0
gi|4377413|emb|CAA36202.1|  open reading frame 1b (AA 1-2733...   634    0.0
gi|133592|sp|P16342|RRPB_CVMA5  RNA-DIRECTED RNA POLYMERASE ...   634    0.0
gi|26008080|ref|NP_150073.2|  orf1ab polyprotein [Bovine cor...   633    e-180
gi|15077820|gb|AAK83365.1|  replicase [bovine coronavirus]        633    e-180
gi|18033972|gb|AAL57305.1|  replicase [bovine coronavirus]        633    e-180
gi|7769353|gb|AAF69342.1|AF208067_2  RNA-directed RNA polyme...   633    e-180
gi|17529672|gb|AAL40397.1|AF220295_2  RNA polymerase 1b [bov...   623    e-177
gi|25121571|ref|NP_740618.1|  coronavirus nsp11 [Murine hepa...   622    e-177
gi|26008092|ref|NP_742140.1|  coronavirus nsp11 [Bovine coro...   617    e-175
gi|10242469|ref|NP_066134.1|  ORF1ab polyprotein; frameshift...   575    e-163
gi|14149033|emb|CAC39112.1|  replicase polyprotein 1ab [Avia...   575    e-163
gi|458735|emb|CAA83018.1|  potential chimeric protein [Avian...   570    e-161
gi|133594|sp|P26314|RRPB_IBVB  RNA-DIRECTED RNA POLYMERASE (...   570    e-161
gi|29293454|gb|AAO67706.1|  ORF1b polyprotein [Avian infecti...   565    e-160
gi|25121555|ref|NP_740631.1|  coronavirus nsp11 [Avian infec...   559    e-158
gi|9635157|ref|NP_058422.1|  replicase [Transmissible gastro...   545    e-153
gi|19387582|ref|NP_598309.1|  Pol1 [porcine epidemic diarrhe...   541    e-152
gi|12175747|ref|NP_073549.1|  replicase polyprotein 1ab [Hum...   535    e-151
gi|133591|sp|P18458|RRPB_BEV  RNA-directed RNA polymerase (O...    50    8e-05
gi|1513061|dbj|BAA13323.1|  cyanoprotein alpha subunit precu...    35    3.7

Alignments


>gi|74827|pir||VFIHJH   genome polyprotein 1b - murine hepatitis virus
(strain JHM)
          Length = 2731

 Score =  638 bits (1645), Expect = 0.0
 Identities = 287/481 (59%), Positives = 366/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
             +TY RLIS+MGFK++   ++GY   +FITR+EAI+ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 1585 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHATRDSIGTNFPLQ 1644

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
             LGFSTG++ V    TG       +    F +   A+ PPG+QFKHL+PLM +G   W+VVRI+IVQ
Sbjct: 1645 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLVPLMSRGQKWDVVRIRIVQ 1704

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
             MLSD L   L+D VV V WA   FELT ++YF K+G E   C +C+KRATCF++ +   Y CW
Sbjct: 1705 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGKEVVCSVCNKRATCFNSRTGYYGCWR 1764

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
             HS    DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1765 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1824
```

```
Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 1825 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1882

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P        +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVLS
Sbjct: 1883 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLSK 1939

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC            +DYVPL
Sbjct: 1940 LNLPGCNGGSLYVNKHAFHTNPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 1999

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2000 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2059

Query: 486  L 486
            L
Sbjct: 2060 L 2060
```

```
>gi|14917044|sp|P29982|RRPB_CVMJH   RNA-directed RNA polymerase (ORF1B)
 gi|7583321|gb|AAA46458.2|   open reading frame 1b [murine hepatitis virus]
          Length = 2731

 Score = 637 bits (1644), Expect = 0.0
 Identities = 287/481 (59%), Positives = 366/481 (76%), Gaps = 5/481 (1%)
```

```
Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FITR+EAI+ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 1585 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHATRDSIGTNFPLQ 1644

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V  TG      +   F +  A+ PPG+QFKHL+PLM +G  W+VVRI+IVQ
Sbjct: 1645 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLVPLMSRGQKWDVVRIRIVQ 1704

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C+KRATCF++ +  Y CW
Sbjct: 1705 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGKEVVCSVCNKRATCFNSRTGYYGCWR 1764

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1765 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1824

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 1825 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1882

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P        +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVLS
Sbjct: 1883 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLSK 1939

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC            +DYVPL
Sbjct: 1940 LNLPGCNGGSLYVNKHAFHTNPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 1999
```

```
Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2000 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2059

Query: 486  L 486
            L
Sbjct: 2060 L 2060
```

```
>gi|26007546|ref|NP_068668.2|   ORF1ab polyprotein [Murine hepatitis virus]
        Length = 7178

 Score =  637 bits (1644), Expect = 0.0
 Identities = 286/481 (59%), Positives = 364/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++   ++GY   +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 6032 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 6091

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG      +    F +   A+ PPG+QFKHLIPLM +G  W+VVRI+IVQ
Sbjct: 6092 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 6151

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA   FELT ++YF K+G E   C +C KRATCF++ +  Y CW
Sbjct: 6152 MLSDHLADLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 6211

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS    DY+YNP ++D+QQWG+TG+L SNHD  C VH   AHVAS DAIMTRCLAVH+CF K
Sbjct: 6212 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 6271

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 6272 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 6329

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P        +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 6330 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 6386

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC            +DYVPL
Sbjct: 6387 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 6446

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 6447 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 6506

Query: 486  L 486
            L
Sbjct: 6507 L 6507
```

```
>gi|7769342|gb|AAF69332.1|AF208066_2   RNA-directed RNA polymerase [murine
hepatitis virus]
         Length = 2732
```

```
 Score =  637 bits (1644), Expect = 0.0
 Identities = 287/481 (59%), Positives = 366/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FITR+EAIR VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 1586 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIRRVRAWVGFDAEGAHATRDSIGTNFPLQ 1645

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG     +   F +  A+ PPG+QFKHL+PLM +G  W+VVRI+IVQ
Sbjct: 1646 LGFSTGIDFVVEATGMFAERDGYVFKKAVARAPPGEQFKHLVPLMSRGQKWDVVRIRIVQ 1705

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C+KRATCF++ +  Y CW
Sbjct: 1706 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGKEVVCSVCNKRATCFNSRTGYYGCWR 1765

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1766 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDLICSVHKGAHVASSDAIMTRCLAVHDCFCK 1825

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+WS+EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 1826 SVNWSLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1883

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P         +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1884 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1940

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC             +DYVPL
Sbjct: 1941 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2000

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA +YR+YL++YN    +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2001 RSATCITRCNLGGAVCLKHAEDYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2060

Query: 486  L 486
            L
Sbjct: 2061 L 2061


>gi|6625761|gb|AAF19384.1|AF201929_2  RNA-directed RNA polymerase [murine
hepatitis virus strain 2]
 gi|7739595|gb|AAF68920.1|AF207902_2  RNA-directed RNA polymerase [murine
hepatitis virus strain ML-11]
          Length = 2733

 Score =  637 bits (1643), Expect = 0.0
 Identities = 287/481 (59%), Positives = 366/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FITR+EAIR VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 1587 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIRRVRAWVGFDAEGAHATRDSIGTNFPLQ 1646

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG     +   F +  A+ PPG+QFKHL+PLM +G  W+VVRI+IVQ
```

```
Sbjct: 1647 LGFSTGIDFVVEATGMFAERDGYVFKKAVARAPPGEQFKHLVPLMSRGQKWDVVRIRIVQ 1706

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C+KRATCF++ +  Y CW
Sbjct: 1707 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGKEVVCSVCNKRATCFNSRTGYYGCWR 1766

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1767 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDLICSVHKGAHVASSDAIMTRCLAVHDCFCK 1826

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+WS+EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV     ++
Sbjct: 1827 SVNWSLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1884

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P       +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1885 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1941

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC          +DYVPL
Sbjct: 1942 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2001

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA +YR+YL++YN    +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2002 RSATCITRCNLGGAVCLKHAEDYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2061

Query: 486  L 486
            L
Sbjct: 2062 L 2062


>gi|2641128|gb|AAB86818.1|   RNA-directed RNA polymerase [murine hepatitis
virus]
          Length = 2733

 Score =  635 bits (1637), Expect = 0.0
 Identities = 286/481 (59%), Positives = 364/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 1587 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 1646

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG      +   F +  A+ PPG+QFKHLIPLM +G  W+VVRI+IVQ
Sbjct: 1647 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 1706

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C KRATCF++ +  Y CW
Sbjct: 1707 MLSDHLADLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 1766

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1767 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1826

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV     ++
```

```
Sbjct: 1827 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1884

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P          +++   Y Y   H D+F  DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1885 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1941

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT   F ++AF NLK +PFFYYSD+PC             +DYVPL
Sbjct: 1942 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2001

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC   HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2002 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2061

Query: 486  L 486
            L
Sbjct: 2062 L 2062


>gi|4377413|emb|CAA36202.1|   open reading frame 1b (AA 1-2733) [Murine
hepatitis virus]
          Length = 2733

 Score =  634 bits (1636), Expect = 0.0
 Identities = 286/481 (59%), Positives = 364/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++   ++GY   +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 1587 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 1646

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG        +     F +  A+ PPG+QFKHLIPLM +G  W+VVRI+IVQ
Sbjct: 1647 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 1706

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E   C +C KRATCF++ +  Y CW
Sbjct: 1707 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 1766

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1767 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1826

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++  ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 1827 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1884

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P          +++   Y Y   H D+F  DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1885 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1941

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT   F ++AF NLK +PFFYYSD+PC             +DYVPL
Sbjct: 1942 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2001

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC   HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFTRLQS
```

```
Sbjct: 2002 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2061

Query: 486  L 486
            L
Sbjct: 2062 L 2062


>gi|133592|sp|P16342|RRPB_CVMA5   RNA-DIRECTED RNA POLYMERASE (ORF1B)
 gi|93916|pir||S15760   genome polyprotein - murine hepatitis virus (strain
A59)
          Length = 2733

 Score =  634 bits (1636), Expect = 0.0
 Identities = 286/481 (59%), Positives = 364/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++   ++GY   +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 1587 VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 1646

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG      +    F +   A+ PPG+QFKHLIPLM +G   W+VVRI+IVQ
Sbjct: 1647 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 1706

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L   L+D VV V WA   FELT ++YF K+G E   C +C KRATCF++ +   Y CW
Sbjct: 1707 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 1766

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS    DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1767 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1826

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV      ++
Sbjct: 1827 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1884

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P        +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1885 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1941

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC          +DYVPL
Sbjct: 1942 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2001

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2002 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2061

Query: 486  L 486
            L
Sbjct: 2062 L 2062


>gi|26008080|ref|NP_150073.2|   orf1ab polyprotein [Bovine coronavirus]
          Length = 7094

 Score =  633 bits (1633), Expect = e-180
```

```
Identities = 284/481 (59%), Positives = 367/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++   ++GY   +FIT+EEA++ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 5948 VTYSRLISLMGFKLDVTLDGYCKLFITKEEAVKRVRAWVGFDAEGAHATRDSIGTNFPLQ 6007

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG      +     F +  AK PPG+QFKHLIPLM +G  W+VVR +IVQ
Sbjct: 6008 LGFSTGIDFVVEATGLFADRDGYSFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQ 6067

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            M +D L  LSD VV V WA  FELT ++YF K+G E +C +C KRAT +++ +  Y CW
Sbjct: 6068 MFADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNVCTKRATAYNSRTGYYGCWR 6127

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HSV  DY+YNP ++D+QQWG+ G+L SNHD +C VH  AHVAS DAIMTRCLAV++CF
Sbjct: 6128 HSVTCDYLYNPLIVDIQQWGYIGSLSSNHDLYCSVHKGAHVASSDAIMTRCLAVYDCFCN 6187

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
            ++W+VEYPII +EL +N++CR +Q +++K+A+L +++ + +DIGNPKAI CV   + ++
Sbjct: 6188 NINWNVEYPIISNELSINTSCRVLQRVMLKAAMLCNRYTLCYDIGNPKAIACV--KDFDF 6245

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDAQP        ++ L YS+  H D F DG+C+FWNCNVD+YP NA+VCRFDTRVL+N
Sbjct: 6246 KFYDAQPI---VKSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNN 6302

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF +LK +PFFYYSD+PC            +DYVPL
Sbjct: 6303 LNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFFYYSDTPCVYMDGMDAKQVDYVPL 6362

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            KSATCITRCNLGGAVC  HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFT+LQS
Sbjct: 6363 KSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTKLQS 6422

Query: 486  L 486
            L
Sbjct: 6423 L 6423


>gi|15077820|gb|AAK83365.1|  replicase [bovine coronavirus]
          Length = 7094

 Score =  633 bits (1633), Expect = e-180
 Identities = 284/481 (59%), Positives = 367/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++   ++GY   +FIT+EEA++ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 5948 VTYSRLISLMGFKLDVTLDGYCKLFITKEEAVKRVRAWVGFDAEGAHATRDSIGTNFPLQ 6007

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG      +     F +  AK PPG+QFKHLIPLM +G  W+VVR +IVQ
Sbjct: 6008 LGFSTGIDFVVEATGLFADRDGYSFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQ 6067

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            M +D L  LSD VV V WA  FELT ++YF K+G E +C +C KRAT +++ +  Y CW
Sbjct: 6068 MFADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNVCTKRATAYNSRTGYYGCWR 6127
```

```
Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HSV  DY+YNP ++D+QQWG+ G+L SNHD +C VH  AHVAS DAIMTRCLAV++CF
Sbjct: 6128 HSVTCDYLYNPLIVDIQQWGYIGSLSSNHDLYCSVHKGAHVASSDAIMTRCLAVYDCFCN 6187

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             ++W+VEYPII +EL +N++CR +Q +++K+A+L +++ + +DIGNPKAI CV   + ++
Sbjct: 6188 NINWNVEYPIISNELSINTSCRVLQRVMLKAAMLCNRYTLCYDIGNPKAIACV--KDFDF 6245

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDAQP         ++ L YS+  H D F DG+C+FWNCNVD+YP NA+VCRFDTRVL+N
Sbjct: 6246 KFYDAQPI---VKSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNN 6302

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF +LK +PFFYYSD+PC            +DYVPL
Sbjct: 6303 LNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFFYYSDTPCVYMDGMDAKQVDYVPL 6362

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            KSATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFT+LQS
Sbjct: 6363 KSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTKLQS 6422

Query: 486  L 486
            L
Sbjct: 6423 L 6423


>gi|18033972|gb|AAL57305.1|  replicase [bovine coronavirus]
          Length = 7094

 Score =  633 bits (1633), Expect = e-180
 Identities = 284/481 (59%), Positives = 367/481 (76%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FIT+EEA++ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 5948 VTYSRLISLMGFKLDVTLDGYCKLFITKEEAVKRVRAWVGFDAEGAHATRDSIGTNFPLQ 6007

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V  TG    +  F + AK PPG+QFKHLIPLM +G  W+VVR +IVQ
Sbjct: 6008 LGFSTGIDFVVEATGLFADRDGYSFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQ 6067

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            M +D L  LSD VV V WA  FELT ++YF K+G E +C +C KRAT +++ +  Y CW
Sbjct: 6068 MFADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNVCTKRATAYNSRTGYYGCWR 6127

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HSV  DY+YNP ++D+QQWG+ G+L SNHD +C VH  AHVAS DAIMTRCLAV++CF
Sbjct: 6128 HSVTCDYLYNPLIVDIQQWGYIGSLSSNHDLYCSVHKGAHVASSDAIMTRCLAVYDCFCN 6187

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             ++W+VEYPII +EL +N++CR +Q +++K+A+L +++ + +DIGNPKAI CV   + ++
Sbjct: 6188 NINWNVEYPIISNELSINTSCRVLQRVMLKAAMLCNRYTLCYDIGNPKAIACV--KDFDF 6245

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDAQP         ++ L YS+  H D F DG+C+FWNCNVD+YP NA+VCRFDTRVL+N
Sbjct: 6246 KFYDAQPI---VKSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNN 6302
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Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF +LK +PFFYYSD+PC          +DYVPL
Sbjct: 6303 LNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFFYYSDTPCVYMDGMDAKQVDYVPL 6362

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            KSATCITRCNLGGAVC  HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFT+LQS
Sbjct: 6363 KSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTKLQS 6422

Query: 486  L 486
            L
Sbjct: 6423 L 6423


>gi|7769353|gb|AAF69342.1|AF208067_2  RNA-directed RNA polymerase [murine
hepatitis virus]
          Length = 2733

 Score =  633 bits (1633), Expect = e-180
 Identities = 285/481 (59%), Positives = 364/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            ++Y RLIS+MGFK++  ++GY  +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 1587 VSYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 1646

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG        +    F +  A+ PPG+QFKHLIPLM +G  W+VVRI+IVQ
Sbjct: 1647 LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 1706

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C KRATCF++ +  Y CW
Sbjct: 1707 MLSDHLVDLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 1766

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 1767 HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 1826

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV     ++
Sbjct: 1827 SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 1884

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDA P       +++   Y Y   H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 1885 KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 1941

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC          +DYVPL
Sbjct: 1942 LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 2001

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            +SATCITRCNLGGAVC  HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFTRLQS
Sbjct: 2002 RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQS 2061

Query: 486  L 486
            L
Sbjct: 2062 L 2062
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>gi|17529672|gb|AAL40397.1|AF220295_2   RNA polymerase 1b [bovine
coronavirus]
          Length = 2685

 Score =  623 bits (1607), Expect = e-177
 Identities = 282/481 (58%), Positives = 365/481 (75%), Gaps = 5/481 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FIT+EEA++ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 1574 VTYSRLISLMGFKLDVTLDGYCKLFITKEEAVKRVRAWVGFDAEGAHATRDSIGTNFPLQ 1633

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
            LGFSTG++ V   TG     +    F +  AK PPG+QFKHLIPLM +G  W+VVR +IVQ
Sbjct: 1634 LGFSTGIDFVVEATGLFADRDGYSFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQ 1693

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
            M +D L   LSD VV V WA   FELT ++YF K+G E +C +   KRAT +++ +   Y CW
Sbjct: 1694 MFADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNVSTKRATAYNSRTGYYGCWR 1753

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
            HSV   DY+YNP ++D+QQWG+ G+L SNHD +C VH  AHVAS DAIMTRCLAV++CF
Sbjct: 1754 HSVTCDYLYNPLIVDIQQWGYIGSLSSNHDLYCSVHKGAHVASSDAIMTRCLAVYDCFCN 1813

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
             ++W+VEYPII +EL +N++CR +Q +++K+A+L +++ + +DIGNPKAI CV   + ++
Sbjct: 1814 NINWNVEYPIISNELSINTSCRVLQRVMLKAAMLCNRYTLCYDIGNPKAIACV--KDFDF 1871

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
            KFYDAQP         ++ L Y +  H D F DG+C+FWNCNVD+YP NA+VCRFDTRVL+N
Sbjct: 1872 KFYDAQPI---VKSVKTLLYFFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNN 1928

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF +LK +PFFYYSD+PC            +DYVPL
Sbjct: 1929 LNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFFYYSDTPCVYMDGMDAKQVDYVPL 1988

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQS 485
            KSATCITRCNLGGAVC  HA EYR+YL++YN    +AGF+ W+YK FD YNLWNTFT+LQS
Sbjct: 1989 KSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTKLQS 2048

Query: 486  L 486
            L
Sbjct: 2049 L 2049


>gi|25121571|ref|NP_740618.1|   coronavirus nsp11 [Murine hepatitis virus]
          Length = 521

 Score =  622 bits (1603), Expect = e-177
 Identities = 284/479 (59%), Positives = 362/479 (75%), Gaps = 5/479 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
            +TY RLIS+MGFK++  ++GY  +FITR+EAI+ VRAW+GFD EG HA RD++GTN PLQ
Sbjct: 48   VTYSRLISLMGFKLDLTLDGYCKLFITRDEAIKRVRAWVGFDAEGAHAIRDSIGTNFPLQ 107

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
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             LGFSTG++ V   TG      +    F +  A+ PPG+QFKHLIPLM +G  W+VVRI+IVQ
Sbjct: 108  LGFSTGIDFVVEATGMFAERDGYVFKKAAARAPPGEQFKHLIPLMSRGQKWDVVRIRIVQ 167

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
             MLSD L  L+D VV V WA  FELT ++YF K+G E  C +C KRATCF++ +  Y CW
Sbjct: 168  MLSDHLADLADSVVLVTWAASFELTCLRYFAKVGREVVCSVCTKRATCFNSRTGYYGCWR 227

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
             HS   DY+YNP ++D+QQWG+TG+L SNHD  C VH  AHVAS DAIMTRCLAVH+CF K
Sbjct: 228  HSYSCDYLYNPLIVDIQQWGYTGSLTSNHDPICSVHKGAHVASSDAIMTRCLAVHDCFCK 287

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
              V+W++EYPII +E+ VN++CR +Q ++ ++A+L +++ V +DIGNPK + CV     ++
Sbjct: 288  SVNWNLEYPIISNEVSVNTSCRLLQRVMFRAAMLCNRYDVCYDIGNPKGLACVKG--YDF 345

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
             KFYDA P         +++  Y Y  H D+F DG+C+FWNCNVD+YPANA+VCRFDTRVL+
Sbjct: 346  KFYDASPV---VKSVKQFVYKYEAHKDQFLDGLCMFWNCNVDKYPANAVVCRFDTRVLNK 402

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
             LNLPGC+GGSLYVNKHAFHT  F ++AF NLK +PFFYYSD+PC            +DYVPL
Sbjct: 403  LNLPGCNGGSLYVNKHAFHTSPFTRAAFENLKPMPFFYYSDTPCVYMEGMESKQVDYVPL 462

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
             +SATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFTRLQ
Sbjct: 463  RSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTRLQ 521
```

```
>gi|26008092|ref|NP_742140.1|   coronavirus nsp11 [Bovine coronavirus]
          Length = 521

 Score =  617 bits (1590), Expect = e-175
 Identities = 282/479 (58%), Positives = 365/479 (76%), Gaps = 5/479 (1%)

Query: 6    MTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQ 65
             +TY RLIS+MGFK++  ++GY  +FIT+EEA++ VRAW+GFD EG HATRD++GTN PLQ
Sbjct: 48   VTYSRLISLMGFKLDVTLDGYCKLFITKEEAVKRVRAWVGFDAEGAHATRDSIGTNFPLQ 107

Query: 66   LGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQ 125
             LGFSTG++ V   TG      +    F +  AK PPG+QFKHLIPLM +G  W+VVR +IVQ
Sbjct: 108  LGFSTGIDFVVEATGLFADRDGYSFKKAVAKAPPGEQFKHLIPLMTRGQRWDVVRPRIVQ 167

Query: 126  MLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWN 185
             M +D L  LSD VV V WA  FELT ++YF K+G E +C +C KRAT +++ +  Y CW
Sbjct: 168  MFADHLIDLSDCVVLVTWAANFELTCLRYFAKVGREISCNVCTKRATAYNSRTGYYGCWR 227

Query: 186  HSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVK 245
             HSV  DY+YNP ++D+QQWG+ G+L SNHD +C VH  AHVAS DAIMTRCLAV++CF
Sbjct: 228  HSVTCDYLYNPLIVDIQQWGYIGSLSSNHDLYCSVHKGAHVASSDAIMTRCLAVYDCFCN 287

Query: 246  RVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEW 305
              ++W+VEYPII +EL +N++CR +Q +++K+A+L +++ + +DIGNPKAI CV   + ++
Sbjct: 288  NINWNVEYPIISNELSINTSCRVLQRVMLKAAMLCNRYTLCYDIGNPKAIACV--KDFDF 345

Query: 306  KFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSN 365
             KFYDAQP        ++ L YS+  H D F DG+C+FWNCNVD+YP NA+VCRFDTRVL+N
```

```
Sbjct: 346  KFYDAQPI---VKSVKTLLYSFEAHKDSFKDGLCMFWNCNVDKYPPNAVVCRFDTRVLNN 402

Query: 366  LNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPL 425
            LNLPGC+GGSLYVNKHAFHT  F ++AF +LK +PFFYYSD+PC          +DYVPL
Sbjct: 403  LNLPGCNGGSLYVNKHAFHTKPFSRAAFEHLKPMPFFYYSDTPCVYMDGMDAKQVDYVPL 462

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            KSATCITRCNLGGAVC  HA EYR+YL++YN   +AGF+ W+YK FD YNLWNTFT+LQ
Sbjct: 463  KSATCITRCNLGGAVCLKHAEEYREYLESYNTATTAGFTFWVYKTFDFYNLWNTFTKLQ 521
```

>gi|10242469|ref|NP_066134.1| ORF1ab polyprotein; frameshift product [Avian infectious bronchitis virus]
Length = 6629

Score = 575 bits (1482), Expect = e-163
Identities = 262/482 (54%), Positives = 344/482 (71%), Gaps = 5/482 (1%)

```
Query: 5     DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
             ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA    +GTNLP
Sbjct: 5515  EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 5574

Query: 65    QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
             Q+GFSTG + V  P G VDT      F  VN+K PPG+QF HL  L      PW+V+R +IV
Sbjct: 5575  QVGFSTGADFVVTPEGLVDTSIGNNFEPVNSKAPPGEQFNHLRVLFKSAKPWHVIRPRIV 5634

Query: 125   QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
             QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 5635  QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 5693

Query: 185   NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
              H +GFD+VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DAIMTRCLA++  F
Sbjct: 5694  KHCLGFDFVYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASVDAIMTRCLAINNAFC 5753

Query: 245   KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
             + V+W + YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 5754  QDVNWDLTYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 5813

Query: 305   WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
             ++FYD  P        + E   Y Y  H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 5814  FRFYDKNPIVRNVKQFE---YDYNQHKDKFADGLCMFWNCNVDCYPDNSLVCRYDTRNLS 5870

Query: 365   NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
               NLPGC+GGSLYVNKHAF+TP FD+ +F NLK +PFF+Y  SPCE+      V+  D V
Sbjct: 5871  VFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 5929

Query: 425   LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
             L +   CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+  + + YNLW +F+ LQ
Sbjct: 5930  LATKDCITKCNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNKLNPYNLWKSFSALQ 5989

Query: 485   SL 486
             S+
Sbjct: 5990  SI 5991
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>gi|14149033|emb|CAC39112.1|   replicase polyprotein 1ab [Avian infectious
bronchitis virus (strain
            Beaudette CK)]
          Length = 6629

 Score =  575 bits (1482), Expect = e-163
 Identities = 262/482 (54%), Positives = 344/482 (71%), Gaps = 5/482 (1%)

Query: 5    DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
            ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA    +GTNLP
Sbjct: 5515 EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 5574

Query: 65   QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
            Q+GFSTG + V  P G VDT      F  VN+K PPG+QF HL  L      PW+V+R +IV
Sbjct: 5575 QVGFSTGADFVVTPEGLVDTSIGNNFEPVNSKAPPGEQFNHLRVLFKSAKPWHVIRPRIV 5634

Query: 125  QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
            QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 5635 QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 5693

Query: 185  NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
             H +GFD+VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DAIMTRCLA++  F
Sbjct: 5694 KHCLGFDFVYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASVDAIMTRCLAINNAFC 5753

Query: 245  KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
            + V+W + YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 5754 QDVNWDLTYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 5813

Query: 305  WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
            ++FYD  P       + E   Y Y  H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 5814 FRFYDKNPIVRNVKQFE---YDYNQHKDKFADGLCMFWNCNVDCYPDNSLVCRYDTRNLS 5870

Query: 365  NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
              NLPGC+GGSLYVNKHAF+TP FD+ +F NLK +PFF+Y  SPCE+     V+  D V
Sbjct: 5871 VFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 5929

Query: 425  LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            L +  CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+  + + YNLW +F+ LQ
Sbjct: 5930 LATKDCITKCNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNKLNPYNLWKSFSALQ 5989

Query: 485  SL 486
            S+
Sbjct: 5990 SI 5991


>gi|458735|emb|CAA83018.1|   potential chimeric protein [Avian infectious
bronchitis virus]
          Length = 2155

 Score =  570 bits (1470), Expect = e-161
 Identities = 262/482 (54%), Positives = 344/482 (71%), Gaps = 5/482 (1%)

Query: 5    DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
            ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA    +GTNLP
Sbjct: 1596 EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 1655
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Query: 65   QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
            Q+GFSTG + V  P G VDT       F  VN+K PPG+QF HL  L      PW+V+R +IV
Sbjct: 1656 QVGFSTGADFVVTPEGLVDTSIGNNFEPVNSKAPPGEQFNHLRVLFKSAKPWHVIRPRIV 1715

Query: 125  QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
            QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 1716 QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 1774

Query: 185  NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
             H +GFD+VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DAIMTRCLA++  F
Sbjct: 1775 KHCLGFDFVYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASVDAIMTRCLAINNAFC 1834

Query: 245  KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
            + V+W + YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 1835 QDVNWDLTYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 1894

Query: 305  WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
            ++FYD  P      + E   Y Y  H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 1895 FRFYDKNPIVRNVKQFE---YDYNQHKDKFADGLCMFWNCNVDCYPDNSLVCRYDTRNLS 1951

Query: 365  NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
              NLPGC+GGSLYVNKHAF+TP FD+ +F NLK +PFF+Y  SPCE+     V+  D V
Sbjct: 1952 VFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 2010

Query: 425  LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            L +  CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+  + + YNLW +F+ LQ
Sbjct: 2011 LATKDCITKCNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNKLNPYNLWKSFSALQ 2070

Query: 485  SL 486
            S+
Sbjct: 2071 SI 2072


>gi|133594|sp|P26314|RRPB_IBVB   RNA-DIRECTED RNA POLYMERASE (ORF1B)
 gi|74826|pir||VFIHB2   genome polyprotein - avian infectious bronchitis
virus  (strain
            Beaudette)
 gi|292953|gb|AAA70234.1|   pol protein [Avian infectious bronchitis virus]
 gi|331173|gb|AAA46224.1|   ORF1b [Avian infectious bronchitis virus]
          Length = 2652

 Score =  570 bits (1469), Expect = e-161
 Identities = 262/482 (54%), Positives = 344/482 (71%), Gaps = 5/482 (1%)

Query: 5    DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
            ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA    +GTNLP
Sbjct: 1538 EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 1597

Query: 65   QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
            Q+GFSTG + V  P G VDT       F  VN+K PPG+QF HL  L      PW+V+R +IV
Sbjct: 1598 QVGFSTGADFVVTPEGLVDTSIGNNFEPVNSKAPPGEQFNHLRVLFKSAKPWHVIRPRIV 1657

Query: 125  QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
            QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 1658 QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 1716
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Query: 185  NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
             H +GFD+VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DAIMTRCLA++  F
Sbjct: 1717 KHCLGFDFVYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASVDAIMTRCLAINNAFC 1776

Query: 245  KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
            + V+W + YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 1777 QDVNWDLTYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 1836

Query: 305  WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
            ++FYD  P       + E   Y Y  H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 1837 FRFYDKNPIVRNVKQFE---YDYNQHKDKFADGLCMFWNCNVDCYPDNSLVCRYDTRNLS 1893

Query: 365  NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
              NLPGC+GGSLYVNKHAF+TP FD+ +F NLK +PFF+Y  SPCE+       V+  D V
Sbjct: 1894 VFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 1952

Query: 425  LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            L +   CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+  + + YNLW +F+ LQ
Sbjct: 1953 LATKDCITKCNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNKLNPYNLWKSFSALQ 2012

Query: 485  SL 486
            S+
Sbjct: 2013 SI 2014
```

>gi|29293454|gb|AAO67706.1| ORF1b polyprotein [Avian infectious bronchitis virus]
Length = 2649

Score = 565 bits (1455), Expect = e-160
Identities = 261/482 (54%), Positives = 342/482 (70%), Gaps = 8/482 (1%)

```
Query: 5    DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
            ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA     +GTNLP
Sbjct: 1538 EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 1597

Query: 65   QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
            Q+GFSTG + V  P G +DT       F VN+K PPG+QF HL  L      PW+V+R +IV
Sbjct: 1598 QVGFSTGADFVVTPEGLIDTSIGNNFEPVNSKAPPGEQFNHLRALFKSAKPWHVIRPRIV 1657

Query: 125  QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
            QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 1658 QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 1716

Query: 185  NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
             H +G   VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DA+MTRCLA++  F
Sbjct: 1717 RHCLG---VYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASADAVMTRCLAINNAFC 1773

Query: 245  KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
            K V+W ++YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 1774 KDVNWELQYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 1833

Query: 305  WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
            ++FYD  P       + E   Y Y+ H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 1834 FRFYDKNPIVPNVKQFE---YDYSQHKDKFADGLCMFWNCNVDCYPENSLVCRYDTRNLS 1890
```

```
Query: 365  NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
              NLPGC+GGSLYVNKHAFHTP FD+ +F NLK +PFF+Y  SPCE+     V+  D V
Sbjct: 1891 VFNLPGCNGGSLYVNKHAFHTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 1949

Query: 425  LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            L +  CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+   F+ YNLW  F+ LQ
Sbjct: 1950 LATKDCITKCNIGGAVCKKHAQMYAEFVFSYNAAVTAGFTFWVTNNFNPYNLWKNFSALQ 2009

Query: 485  SL 486
            S+
Sbjct: 2010 SI 2011


>gi|25121555|ref|NP_740631.1|   coronavirus nsp11 [Avian infectious
bronchitis virus]
          Length = 521

 Score =  559 bits (1440), Expect = e-158
 Identities = 261/480 (54%), Positives = 342/480 (71%), Gaps = 5/480 (1%)

Query: 5    DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPL 64
            ++TY+ LIS++GFKM+  V G  NMFITR+EAIR+VR W+GFDVE  HA    +GTNLP
Sbjct: 47   EITYKHLISLLGFKMSVNVEGCHNMFITRDEAIRNVRGWVGFDVEATHACGTNIGTNLPF 106

Query: 65   QLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIV 124
            Q+GFSTG + V  P G VDT     F  VN+K PPG+QF HL  L     PW+V+R +IV
Sbjct: 107  QVGFSTGADFVVTPEGLVDTSIGNNFEPVNSKAPPGEQFNHLRVLFKSAKPWHVIRPRIV 166

Query: 125  QMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACW 184
            QML+D L  +SD VVFV W HG ELT+++YFVKIG E+ C  C  RAT F++ +  YACW
Sbjct: 167  QMLADNLCNVSDCVVFVTWCHGLELTTLRYFVKIGKEQVCS-CGSRATTFNSHTQAYACW 225

Query: 185  NHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFV 244
             H +GFD+VYNP ++D+QQWG++GNLQ NHD HC VHG+AHVAS DAIMTRCLA++  F
Sbjct: 226  KHCLGFDFVYNPLLVDIQQWGYSGNLQFNHDLHCNVHGHAHVASVDAIMTRCLAINNAFC 285

Query: 245  KRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVE 304
            + V+W + YP I +E  VNS+CR +Q M + + + A K  V++DIGNPK IKCV + +V
Sbjct: 286  QDVNWDLTYPHIANEDEVNSSCRYLQRMYLNACVDALKVNVVYDIGNPKGIKCVRRGDVN 345

Query: 305  WKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLS 364
            ++FYD  P      + E   Y Y  H DKF DG+C+FWNCNVD YP N++VCR+DTR LS
Sbjct: 346  FRFYDKNPIVRNVKQFE---YDYNQHKDKFADGLCMFWNCNVDCYPDNSLVCRYDTRNLS 402

Query: 365  NLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVP 424
              NLPGC+GGSLYVNKHAF+TP FD+ +F NLK +PFF+Y  SPCE+     V+  D V
Sbjct: 403  VFNLPGCNGGSLYVNKHAFYTPKFDRISFRNLKAMPFFFYDSSPCETIQVDGVAQ-DLVS 461

Query: 425  LKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRLQ 484
            L +  CIT+CN+GGAVC+ HA  Y +++ +YN  ++AGF+ W+  + + YNLW +F+ LQ
Sbjct: 462  LATKDCITKCNIGGAVCKKHAQMYAEFVTSYNAAVTAGFTFWVTNKLNPYNLWKSFSALQ 521


>gi|9635157|ref|NP_058422.1|   replicase [Transmissible gastroenteritis
virus]
 gi|7801348|emb|CAB91143.1|   replicase [Transmissible gastroenteritis virus]
```

```
        Length = 6685

 Score =  545 bits (1403), Expect = e-153
 Identities = 261/484 (53%), Positives = 335/484 (69%), Gaps = 13/484 (2%)

Query: 4    KDMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLP 63
            KD+ Y  +IS MGF+    + GY  +F TR+ A+R+VRAW+GFDVEG H   D VGTN+P
Sbjct: 5574 KDVKYANVISYMGFRFEANIPGYHTLFCTRDFAMRNVRAWLGFDVEGAHVCGDNVGTNVP 5633

Query: 64   LQLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKI 123
            LQLGFS GV+ V    G V TE         V A+ PPG+QF HLIPLM KG PW++VR +I
Sbjct: 5634 LQLGFSNGVDFVVQTEGCVITEKGNSIEVVKARAPPGEQFAHLIPLMRKGQPWHIVRRRI 5693

Query: 124  VQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIG-PERTCCLCDKRATCFSTSSDTYA 182
            VQM+ D   GLSD ++FVLWA G ELT+M+YFVKIG P++  C C K ATC+S+S   YA
Sbjct: 5694 VQMVCDYFDGLSDILIFVLWAGGLELTTMRYFVKIGRPQK--CECGKSATCYSSSQSVYA 5751

Query: 183  CWNHSVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHEC 242
            C+ H++G DY+YNP+ ID+QQWG+TG+L  NH + C +H N HVAS DAIMTRCLA+H+C
Sbjct: 5752 CFKHALGCDYLYNPYCIDIQQWGYTGSLSMNHHEVCNIHRNEHVASGDAIMTRCLAIHDC 5811

Query: 243  FVKRVDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAE 302
            FVKRVDWS+ YP I +E ++N A R VQ  V+K+AL        +HD+GNPK I+C
Sbjct: 5812 FVKRVDWSIVYPFIDNEEKINKAGRIVQSHVMKAALKIFNPAAIHDVGNPKGIRCA-TTP 5870

Query: 303  VEWKFYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRV 362
            + W  YD  P ++    +  L Y Y +H      +G+ LFWNCNVD YP  +IVCRFDTR
Sbjct: 5871 IPWFCYDRDPINN---NVRCLDYDYMVHGQ--MNGLMLFWNCNVDMYPEFSIVCRFDTRT 5925

Query: 363  LSNLNLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDY 422
             S L+L GC+GG+LYVN HAFHTPA+D+ AF  LK +PFFYY DS CE     V    +Y
Sbjct: 5926 RSKLSLEGCNGGALYVNNHAFHTPAYDRRAFAKLKPMPFFYYDDSNCE----LVDGQPNY 5981

Query: 423  VPLKSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTR 482
            VPLKS  CIT+CN+GGAVC+ HA  YR Y++ YN+ + AGF++ W  + FDTY LW+ F
Sbjct: 5982 VPLKSNVCITKCNIGGAVCKKHAALYRAYVEDYNIFMQAGFTIWCPQNFDTYMLWHGFVN 6041

Query: 483  LQSL 486
            ++L
Sbjct: 6042 SKAL 6045


>gi|19387582|ref|NP_598309.1|  Pol1 [porcine epidemic diarrhea virus]
 gi|13752450|gb|AAK38661.1|  Pol1 [porcine epidemic diarrhea virus]
        Length = 6781

 Score =  541 bits (1394), Expect = e-152
 Identities = 256/480 (53%), Positives = 334/480 (69%), Gaps = 12/480 (2%)

Query: 8    YRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQLG 67
            Y  +IS MGF+ +  +  +  +F TR+ A+R+VR W+GFDVEG H     VGTN+PLQLG
Sbjct: 5675 YEHVISFMGFRFDINIPNHHTLFCTRDFAMRNVRGWLGFDVEGAHVVGSNVGTNVPLQLG 5734

Query: 68   FSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQML 127
            FS GV+ V  P G V TE+      V A+ PPG+QF HL+PL+ +G PW+VVR +IVQM
Sbjct: 5735 FSNGVDFVVRPEGCVVTESGDYIKPVRARAPPGEQFAHLLPLLKRGQPWDVVRKRIVQMC 5794
```

```
Query: 128  SDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWNHS 187
            SD L  LSD ++FVLWA G ELT+M+YFVKIGP ++C  C K ATC++++  TY C+ H+
Sbjct: 5795 SDYLANLSDILIFVLWAGGLELTTMRYFVKIGPSKSCD-CGKVATCYNSALHTYCCFKHA 5853

Query: 188  VGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVKRV 247
            +G DY+YNP+ ID+QQWG+ G+L  NH +HC VH N HVAS DAIMTRCLA+H+CFVK V
Sbjct: 5854 LGCDYLYNPYCIDIQQWGYKGSLSLNHHEHCNVHRNEHVASGDAIMTRCLAIHDCFVKNV 5913

Query: 248  DWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEWKF 307
            DWS+ YP IG+E  +N + R VQ   ++S L        ++DIGNPK I+C     + +W
Sbjct: 5914 DWSITYPFIGNEAVINKSGRIVQSHTMRSVLKLYNPKAIYDIGNPKGIRCA-VTDAKWFC 5972

Query: 308  YDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSNLN 367
            +D  P +      +E   Y Y I H +F DG+CLFWNCNVD YP  ++VCRFDTR  S LN
Sbjct: 5973 FDKNPTNSNVKTLE---YDY-ITHGQF-DGLCLFWNCNVDMYPEFSVVCRFDTRCRSPLN 6027

Query: 368  LPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKS 427
            L GC+GGSLYVN HAFHTPAFDK AF  LK +PFF+Y D+ C+      ++    I+YVPL++
Sbjct: 6028 LEGCNGGSLYVNNHAFHTPAFDKRAFAKLKPMPFFFYDDTECD----KLQDSINYVPLRA 6083

Query: 428  ATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFT-RLQSL 486
            + CIT+CN+GGAVC  H   Y  Y++AYN   SAGF++W+  FDTYNLW TF+  LQ L
Sbjct: 6084 SNCITKCNVGGAVCSKHCAMYHSYVNAYNTFTSAGFTIWVPTSFDTYNLWQTFSNNLQGL 6143
```

>gi|12175747|ref|NP_073549.1| replicase polyprotein 1ab [Human coronavirus 229E]
gi|12082740|gb|AAG48591.1|AF304460_2 replicase polyprotein 1ab [Human coronavirus 229E]
Length = 6758

Score = 535 bits (1379), Expect = e-151
Identities = 254/478 (53%), Positives = 329/478 (68%), Gaps = 13/478 (2%)

```
Query: 7    TYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLPLQL 66
            TY   +IS MGF+ +   + G   ++F TR+ A+RHVR W+G DVEG H T D VGTN+PLQ+
Sbjct: 5642 TYEHVISYMGFRFDVSMPGSHSLFCTRDFAMRHVRGWLGMDVEGAHVTGDNVGTNVPLQV 5701

Query: 67   GFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQM 126
            GFS GV+ VA P G V T    +      V A+ PPG+QF H++PL+ KG PW+V+R +IVQM
Sbjct: 5702 GFSNGVDFVAQPEGCVLTNTGSVVKPVRARAPPGEQFTHIVPLLRKGQPWSVLRKRIVQM 5761

Query: 127  LSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYACWNH 186
            ++D L G SD +VFVLWA G ELT+M+YFVKIG   +  C C   ATC+++ S+ Y C+ H
Sbjct: 5762 IADFLAGSSDVLVFVLWAGGLELTTMRYFVKIGAVKH-CQCGTVATCYNSVSNDYCCFKH 5820

Query: 187  SVGFDYVYNPFMIDVQQWGFTGNLQSNHDQHCQVHGNAHVASCDAIMTRCLAVHECFVKR 246
            ++G DYVYNP++ID+QQWG+ G+L +NH   C VH N HVAS DAIMTRCLAV++CFVK
Sbjct: 5821 ALGCDYVYNPYVIDIQQWGYVGSLSTNHHAICNVHRNEHVASGDAIMTRCLAVYDCFVKN 5880

Query: 247  VDWSVEYPIIGDELRVNSACRKVQHMVVKSALLADKFPVLHDIGNPKAIKCVPQAEVEWK 306
            VDWS+ YP+I +E  +N  R VQ ++++A+        +HDIGNPK I+C     + +W
Sbjct: 5881 VDWSITYPMIANENAINKGGRTVQSHIMRAAIKLYNPKAIHDIGNPKGIRCA-VTDAKWY 5939

Query: 307  FYDAQPCSDKAYKIEELFYSYAIHHDKFTDGVCLFWNCNVDRYPANAIVCRFDTRVLSNL 366
```

```
                YD  P +      +E   Y Y  H       DG+CLFWNCNVD YP  +IVCRFDTR  S L
Sbjct: 5940 CYDKNPINSNVKTLE---YDYMTHGQ--MDGLCLFWNCNVDMYPEFSIVCRFDTRTRSTL 5994

Query: 367  NLPGCDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSDSPCESHGKQVVSD-IDYVPL 425
             NL G +GGSLYVN HAFHTPA+DK A    LK  PFFYY D  CE       VV D ++YVPL
Sbjct: 5995 NLEGVNGGSLYVNNHAFHTPAYDKRAMAKLKPAPFFYYDDGSCE-----VVHDQVNYVPL 6049

Query: 426  KSATCITRCNLGGAVCRHHANEYRQYLDAYNMMISAGFSLWIYKQFDTYNLWNTFTRL 483
             ++   CIT+CN+GGAVC   HAN YR Y+++YN+    AGF++W+    FD YNLW TFT +
Sbjct: 6050 RATNCITKCNIGGAVCSKHANLYRAYVESYNIFTQAGFNIWVPTTFDCYNLWQTFTEV 6107


>gi|133591|sp|P18458|RRPB_BEV   RNA-directed RNA polymerase (ORF1B)
 gi|94017|pir||S11238   polymerase - Berne virus
 gi|1334814|emb|CAA36601.1|   2nd polymerase reading frame (AA 1-2291) [Berne
virus]
          Length = 2291

 Score = 50.1 bits (118), Expect = 8e-05
 Identities = 37/103 (35%), Positives = 54/103 (52%), Gaps = 11/103 (10%)

Query: 140  FVLWAHGFELTSMKYFVKIGPERTC--CLCDKRATCFSTSSDTYACWNHSVGF--DYVYN 195
             F+L++     +L  S+K++V++       TC   C  C + A C      + Y C N    G       + N
Sbjct: 1511 FILYSCSNDLKSLKFYVEFD---TCYFCSCGEMAICLMRDGN-YKCRNCYGGMLISKLVN 1566

Query: 196  PFMIDVQQWGFTGNLQSNHDQHC-QVHGNAHVASCDAIMTRCL 237
                 +DVQ+        LQ  HD  C Q HG++H A CDA+MT+CL
Sbjct: 1567 CKYLDVQKERV--KLQDAHDAICQQFHGDSHEALCDAVMTKCL 1607


>gi|1513061|dbj|BAA13323.1|   cyanoprotein alpha subunit precursor [Riptortus
clavatus]
          Length = 693

 Score = 34.7 bits (78), Expect = 3.7
 Identities = 16/36 (44%), Positives = 22/36 (61%), Gaps = 1/36 (2%)

Query: 371 CDGGSLYVNKHAFHTPAFDKSAFTNLKQLPFFYYSD 406
            C G  LY +KHA    P FD+ A+ +  Q+P FY+ D
Sbjct: 643 CGGSKLYDSKHAMGFP-FDRPAYPDAFQVPNFYFKD 677


  Database: All non-redundant GenBank CDS
  translations+PDB+SwissProt+PIR+PRF
    Posted date:  Apr 11, 2003  2:30 AM
  Number of letters in database: 454,141,287
  Number of sequences in database:  1,411,415

Lambda     K      H
   0.325    0.139    0.456

Gapped
Lambda     K      H
   0.267    0.0410    0.140
```

```
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 473,361,261
Number of Sequences: 1411415
Number of extensions: 20503315
Number of successful extensions: 51018
Number of sequences better than 10.0: 27
Number of HSP's better than 10.0 without gapping: 26
Number of HSP's successfully gapped in prelim test: 1
Number of HSP's that attempted gapping in prelim test: 50937
Number of HSP's gapped (non-prelim): 33
length of query: 486
length of database: 454,141,287
effective HSP length: 127
effective length of query: 359
effective length of database: 274,891,582
effective search space: 98686077938
effective search space used: 98686077938
T: 11
A: 40
X1: 15 ( 7.0 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 40 (21.6 bits)
S2: 75 (33.5 bits)
```

FIGURE 125

FIGURE 125A

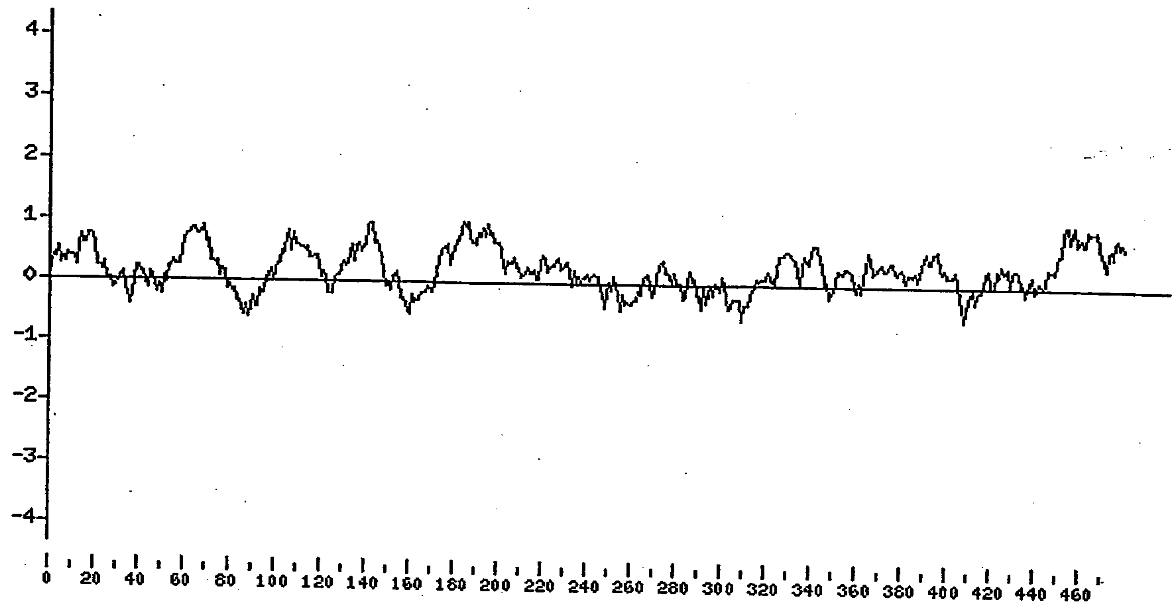


FIGURE 125B

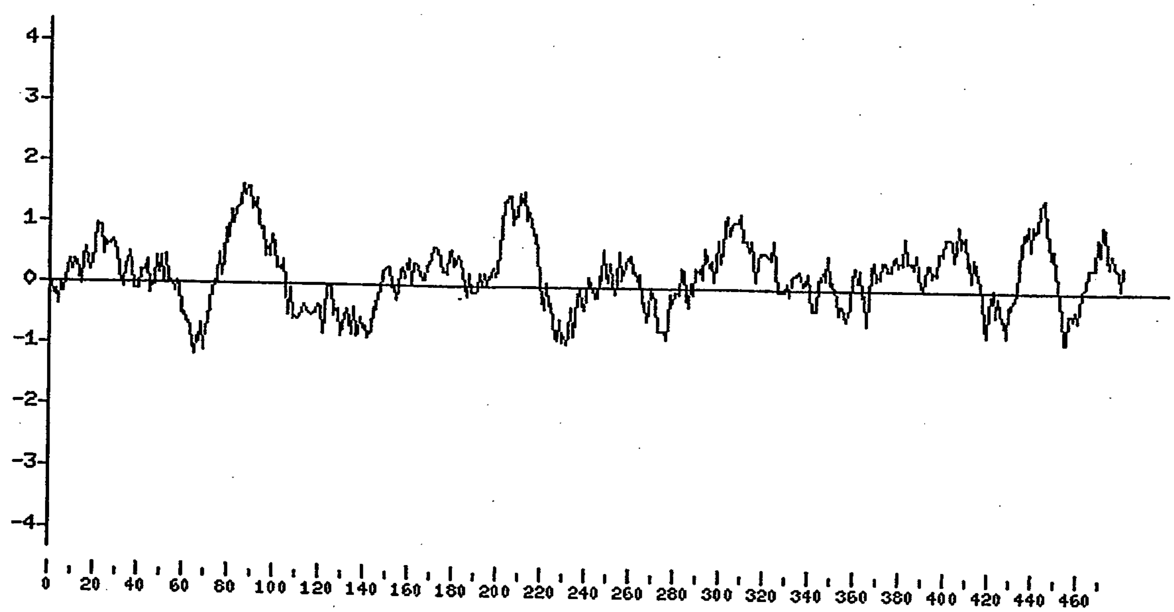


FIGURE 126

5'3' Frame 1

QVHQNVCVL-LIFYLMTLSR--SHKICQ-FQKWSRLQLTMLKFHSCFGVRMDMLKPSTQN
YKQVKRGNQVLRCLTCTRCKECFLKSVTFRIMVKMLLYQKE---MSQSILNCVNT-IHLL
-LYPPT-ELFTLVL

5'3' Frame 2

RFIKMCVFCD-SFT--LCRDNKVTRFVSDFKSGQGYN-LC-NFIHALV-GWTC-NLLPKT
TSKSSVATRCCDA-LVQDAKNAS-KV-PSELW-KCCYTKRNNDECRKVYSTVSILKYTYF
SCTLQHESYSLWCW

5'3' Frame 3

GSSKCVCSVIDLLLDDFVEIIKSQDLSVISKVVKVTIDYAEISFMLWCKDGHVETFYPKL
QASQAWQPGVAMPNLYKMQRMLLEKCDLQNYGENAVIPKGIMMNVAKYTQLCQYLNTLTL
AVPSNMRVIHFGAG

3'5' Frame 1

PAPK-ITLMLEGTAKVSVFKY-HS-VYFATFIIIPFGITAFSP-F-RSHFSRSILCILYK
LGIATPGCHA-LACSFG-KVSTCPSLHQSMNEISA-SIVTLTTFEITDKSCDFIISTKSS
SKRSITEHTHFDEP

3'5' Frame 2

QHQSE-LSCWRVQLK-VYLSIDTVEYTLRHSSLFLLV-QHFHHNSEGHTFQEAFFASCTS
-ASQHLVATLDLLVVLGRRFQHVHPYTKA-MKFQHSQL-P-PLLKSLTNLVTLLSRQSHQ
VKDQSQNTHILMNL

3'5' Frame 3

STKVNNSHVGGYS-SKCI-VLTQLSILCDIHHYSFWYNSIFTIILKVTLFKKHSLHLVQV
RHRNTWLPRLTCL-FWVEGFNMSILTPKHE-NFSIVNCNLDHF-NH-QIL-LYYLDKVIK
-KINHRTHTF—T

FIGURE 127

5'3' Frame 1

-VFTYPGKANQPRSLVDLFSKRTN-NV--WTPIKPT-CPPHYIWWTHRFN-Q-PEWRTAM
GQGQNSADPKVYPIILRLGSQLSLSMARRNLDSLEARAFQSTPIVVQMTKLATTEELPDE
FVVVTAK-KSSAPDGTSIT-ELAQKLHFPTALTKKASYGLQLREP-IHPKTTLAPAILIT
MLPPCYNFLKEQHCQKASTQREAEAAVKPLLAPHHVVAVIQEIQLLAAVGEILLLEWLAE
VVKLPSRYCC-TD-TSLRAKFLVKANNNKAKLSLRNLLLRHLKSLAKNVLPQNSTTSLKH
LGDVVQNKPKEISGTKT-SDKELITNIGPQIAQFA

5'3' Frame 2

RFLPTQEKPTNLDLL-ICSLNEQIKMSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGLQW
GKAKTAPTPRFTQ-YCVLVHSSHSAWQGGT-IPSRPGRSNQHQ-WSR-PNWLLPKSYPTS
SWW-RQNERAQPQMVLLLPRNWPRSFTSLRR-QRRHRMGCN-GSLEYTQRPHWHPQS--Q
CCHRATTSSRNNIAKRLLRRGKQRRQSSLFSLLIT-SR-FKKFNSWQQ-GKFSCSNG-RR
W-NCPRAIAARQIEPA-EQSFW-RPTTTRPNCH-EICC-GI-KASPKTYCHKTVQRHSSI
WETWSRTNPRKFRGPRPNQTRN-LQTLGRKLHNLP

5'3' Frame 3

GFYLPRKSQPTSISCRSVL-TNKLKCLIMDPNQTNVVPPALHLVDPQIQLTITRMEDCNG
ARPKQRRPQGLPNNIASWFTALTQHGKEELRFPRGQGVPINTNSGPDDQIGYYRRATRRV
RGGDGKMKELSPRWYFYYLGTGPEASLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNN
AATVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGG
GETALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAF
GRRGPEQTQGNFGDQDLIRQGTDYKHWAANCTIC

3'5' Frame 1

RQIVQFAAQCL-SVPCLIRSWSPKFPWVCSGPRLPNA-VTLYCFVAVRFWRGFLDASAAD
FLVTVWPCCCWPLPETLLSSWFNLSSSNSARAVSPPPLAIRAGEFPLLLPGVEFLELPRL
RDEEREEA-LPPLLPSA-KPFGNVVP-GSCSTVAALLLGLRVPMWSLGVFKAPSVATHTM
PSLLAP-GSEASGPVPR--KYHLGLSSFILPSPPRTRRVALR--PIWSSGPLLVLIGTPW
PRGNLSSSLPC-VRAVNQDAILLGKPWGRRCFGLAPLQSSILVIVS-ICGSTKCNAGGTT
LV-LGSIIRHFNLFV-RTDLQEIEVGWLFLGR-KP

3'5' Frame 2

GKLCNLRPNVCNQFLV-LGLGPRNFLGFVLDHVSQMLE-RCTVLWQYVFGEAF-MPQQQI

S--QFGLVVVGLYQKLCSQAGSICLAAIARGQFHHLR-PFEQENFPYCCQELNFLNYRDY
VMRSEKRLDCRLCFPLRRSLLAMLFLEEVVARWQHCY-DCGCQCGLWVYSRLPQLQPIRC
LLC-RRREVKLLGQFLGNRSTIWG-ALSFCRHHHELVG-LFGSSQFGHLDHYWC-LERPG
LEGI-VPPCHAE-EL-TKTQYYWVNLGVGAVLALPHCSPPFWLLSVESVGPPNVMRGALR
WFDWGPLSDILICSFREQIYKRSRLVGFSWVGKNL

3'5' Frame 3

ANCAICGPMFVISSLSD-VLVPEISLGLFWTTSPKCLSDVVLFCGSTFLARLFRCLSSRF
LSDSLALLLLAFTRNFALKLVQSV-QQ-REGSFTTSASHSSRRISPTAARS-IS-ITATT
--GARRGLTAASASLCVEAFWQCCSLRKL-HGGSIVIRIAGANVVFGCIQGSLSCNPYDA
FFVSAVGK-SFWASS-VIEVPSGAELFHFAVTTTNSSGSSSVVANLVIWTTIGVDWNALA
SRESKFLLAMLSESCEPRRNIIG-TLGSALFWPCPIAVLHSGYCQLNLWVHQM-CGGHYV
GLIGVHYQTF-FVRLENRSTRDRGWLAFPG-VKT

FIGURE 128

-GLELKL-LTSICAF-PFCYSLF--CLLYFGFHSKSRI-KNLVPKSKRT-NFSLF-LVFL
YAVAYAL-YSAVHLINLMCLKILVRYNTRGNTYSTAWLCALGKVLPFHRWHTMVQTCTPN
VTINCQDPAGGALIARCWYLHEGHQTAAFRDVLVVLNKRTN-NV--WTPIKPT-CPPHYI
WWTHRFN-Q-PEWRTQWGKAKTAPTPRFTQ-YCVLVHSSHSAWQGGT-IPSRPGRSNQHQ
-WSR-PNWLLPKSYPTSSWW-RQNERAQPQMVLLLPRNWPRSFTSLRR-QRRHRMGCN-G
SLEYTQRPHWHPQS--QCCHRATTSSRNNIAKRLLRRGKQRRQSSLFSLLIT-SR-FKKF
NSWQQ-GKFSCSNG-RRW-NCPRAIAARQIEPA-EQSFW-RPTTTRPNCH-EICC-GI-K
ASPKTYCHKTVQRHSSIWETWSRTNPRKFRGPRPNQTRN-LQTLAANCTICSKCLCILWN
VTHWHGSHTFGNMADLSWSH-IG-QRSTIQRQRHTAEQAH-RIQNIPTNRA-KGQKEKD-
-SSAFAAETKEAAHCDSSSC

EDSSSSFN-LLFVLFSLSAIPCFNNAYYILVFTRNPGSRRTLYQSLNEHETSHCFDLYFS
MQLHMHCSTALCI--TSCA-RSL-GTTLGVILIALLGFVL-ERFYLFIDGTLWFKHAHLM
LLSTVKIQLVVRL-LGVGTFMKVTKLLHLETYLLF-INEQIKMSDNGPQSNQRSAPRITF
GGPTDSTDNNQNGGRNGARPKQRRPQGLPNNIASWFTALTQHGKEELRFPRGQGVPINTN
SGPDDQIGYYRRATRRVRGGDGKMKELSPRWYFYYLGTGPEASLPYGANKEGIVWVATEG
ALNTPKDHIGTRNPNNNAATVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRGNSRNS
TPGSSRGNSPARMASGGGETALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKK
PRQKRTATKQYNVTQAFGRRGPEQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGM
SRIGMEVTPSGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKKKKTD
EAQPLPQRQKKQPTVTLLP

RTRAQALIDFYLCFLAFLLFLVLIMLIIFWFSLEIQDLEEPCTKV-TNMKLLIVLTCISL
CSCICTVVQRCASNKPHVLEDPCKVQH-G-YL-HCLALCSRKGFTFS-MAHYGSNMHT-C
YYQLSRSSWWCAYS-VLVPS-RSPNCCI-RRTCCFK-TNKLKCLIMDPNQTNVVPPALHL
VDPQIQLTITRMEDAMGQGQNSADPKVYPIILRLGSQLSLSMARRNLDSLEARAFQSTPI
VVQMTKLATTEELPDEFVVVTAK-KSSAPDGTSIT-ELAQKLHFPTALTKKASYGLQLRE
P-IHPKTTLAPAILITMLPPCYNFLKEQHCQKASTQREAEAAVKPLLAPHHVVAVIQEIQ
LLAAVGEILLLEWLAEVVKLPSRYCC-TD-TSLRAKFLVKANNNKAKLSLRNLLLRHLKS
LAKNVLPQNSTTSLKHLGDVVQNKPKEISGTKT-SDKELITNIGRKLHNLLQVPLHSLEC
HALAWKSHLREHG-LIMEPLNWMTKIHNSKTTSYC-TSTLTHTKHSHQQSLKRTKRKRLM
KLSLCRRDKRSSPL-LFFL

FIGURE 129

5'3' Frame 1

taccgtagactcatctctatgatgggtttcaaaatgaattaccaagtcaatggttaccct
Y R R L I S M M G F K M N Y Q V N G Y P
aatatgtttatcacccgcgaagaagctattcgtcacgttcgtgcgtggattggctttgat
N M F I T R E E A I R H V R A W I G F D
gtagagggctgtcatgcaactagagatgctgtgggtactaacctacctctccagctagga
V E G C H A T R D A V G T N L P L Q L G
ttttctacaggtgttaacttagtagctgtaccgactggttatgttgacactgaaaataac
F S T G V N L V A V P T G Y V D T E N N
acagaattcaccagagttaatgcaaaacctccaccaggtgaccagtttaaacatcttatacc
T E F T R V N A K P P P G D Q F K H L I

5'3' Frame 2

taccgtagactcatctctatgatgggtttcaaaatgaattaccaagtcaatggttaccta
T V D S S L - W V S K - I T K S M V T L
atatgtttatcacccgcgaagaagctattcgtcacgttcgtgcgtggattggctttgatg
I C L S P A K K L F V T F V R G L A L M
tagagggctgtcatgcaactagagatgctgtgggtactaacctacctctccagctaggat
- R A V M Q L E M L W V L T Y L S S - D
tttctacaggtgttaacttagtagctgtaccgactggttatgttgacactgaaaataaca
F L Q V L T - - L Y R L V M L T L K I T
cagaattcaccagagttaatgcaaaacctccaccaggtgaccagtttaaacatcttatacc
Q N S P E L M Q N L H Q V T S L N I L Y

5'3' Frame 3

taccgtagactcatctctatgatgggtttcaaaatgaattaccaagtcaatggttaccctaa
P - T H L Y D G F Q N E L P S Q W L P -
tatgtttatcacccgcgaagaagctattcgtcacgttcgtgcgtggattggctttgatgt
Y V Y H P R R S Y S S R S C V D W L - C
agagggctgtcatgcaactagagatgctgtgggtactaacctacctctccagctaggatt
R G L S C N - R C C G Y - P T S P A R I
ttctacaggtgttaacttagtagctgtaccgactggttatgttgacactgaaaataacac
F Y R C - L S S C T D W L C - H - K - H
agaattcaccagagttaatgcaaaacctccaccaggtgaccagtttaaacatcttatacc
R I H Q S - C K T S T R - P V - T S Y T

3'5' Frame 1

ggtataagatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattct
G I R C L N W S P G G G F A L T L V N S
gtgttattttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaa
V L F S V S T - P V G T A T K L T P V E
aatcctagctggagaggtaggttagtacccacagcatctctagttgcatgacagccctct
N P S W R G R L V P T A S L V A - Q P S
acatcaaagccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacata
T S K P I H A R T - R I A S S R V I N I
ttagggtaaccattgacttggtaattcattttgaaacccatcatagagatgagtctacggta
L G - P L T W - F I L K P I I E M S L R

3'5' Frame 2

ggtataagatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattctg
V - D V - T G H L V E V L H - L W - I L
tgttattttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaaa
C Y F Q C Q H N Q S V Q L L S - H L - K
atcctagctggagaggtaggttagtacccacagcatctctagttgcatgacagccctcta
I L A G E V G - Y P Q H L - L H D S P L
catcaaagccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacatat
H Q S Q S T H E R D E - L L R G - - T Y
tagggtaaccattgacttggtaattcattttgaaacccatcatagagatgagtctacggta
- G N H - L G N S F - N P S - R - V Y G

3'5' Frame 3

```
ggtataagatgtttaaactggtcacctggtggaggttttgcattaactctggtgaattctgt
  Y  K  M  F  K  L  V  T  W  W  R  F  C  I  N  S  G  E  F  C
gttattttcagtgtcaacataaccagtcggtacagctactaagttaacacctgtagaaaa
 V  I  F  S  V  N  I  T  S  R  Y  S  Y  -  V  N  T  C  R  K
tcctagctggagaggtaggttagtacccacagcatctctagttgcatgacagccctctac
 S  -  L  E  R  -  V  S  T  H  S  I  S  S  C  M  T  A  L  Y
atcaaagccaatccacgcacgaacgtgacgaatagcttcttcgcgggtgataaacatatt
 I  K  A  N  P  R  T  N  V  T  N  S  F  F  A  G  D  K  H  I
agggtaaccattgacttggtaattcatttgaaacccatcatagagatgagtctacggta
 R  V  T  I  D  L  V  I  H  F  E  T  H  H  R  D  E  S  T  V
```

FIGURE 130

```
                             10        20        30        40        50        60
                              |         |         |         |         |         |
SEQ ID NO: 9997  KGHDLRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLP
SEQ ID NO:10034  ----YRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLP
                     ********************************************************
Prim. Cons.      KGHD2RRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATRDAVGTNLP

                             70        80        90       100       110       120
                              |         |         |         |         |         |
SEQ ID NO: 9997  LQLGFSTGVNLVAVPTGYVDTENNTKFTRVNAQTSTSEQFKHLIPLMYKGLPWNVVRIKI
SEQ ID NO:10034  LQLGFSTGVNLVAVPTGYVDTENNTEFTRVNAKPPPGDQFKHLI----------------
                 ************************:******:....:******
Prim. cons.      LQLGFSTGVNLVAVPTGYVDTENNT2FTRVNA222222QFKHLIPLMYKGLPWNVVRIKI

                            130       140       150       160       170       180
                              |         |         |         |         |         |
SEQ ID NO: 9997  VQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYAC
SEQ ID NO:10034  ------------------------------------------------------------

Prim. cons.      VQMLSDTLKGLSDRVVFVLWAHGFELTSMKYFVKIGPERTCCLCDKRATCFSTSSDTYAC

                            190       200
                              |         |
SEQ ID NO: 9997  WNHSVGFDYVYNPFMIDVQQWGLYG
SEQ ID NO:10034  -------------------------
```

FIGURE 131

5'3' Frame 1

```
caggttcatcaaaatgtgtgtgttctgtgattgatcttttacttgatgactttgtcgaga
 Q  V  H  Q  N  V  C  V  L  -  L  I  F  Y  L  M  T  L  S  R
taataaagtcacaagatttgtcagtgatttcaaaagtggtcaaggttacaattgactatg
 -  -  S  H  K  I  C  Q  -  F  Q  K  W  S  R  L  Q  L  T  M
ctgaaatttcattcatgctttggtgtaaggatggacatgttgaaaccttctacccaaaac
 L  K  F  H  S  C  F  G  V  R  M  D  M  L  K  P  S  T  Q  N
tacaagcaagtcaagcgtggcaaccaggtgttgcgatgcctaacttgtacaagatgcaaa
 Y  K  Q  V  K  R  G  N  Q  V  L  R  C  L  T  C  T  R  C  K
gaatgcttcttgaaaagtgtgaccttcagaattatggtgaaaatgctgttataccaaaag
 E  C  F  L  K  S  V  T  F  R  I  M  V  K  M  L  L  Y  Q  K
gaataatgatgaatgtcgcaaagtatactcaactgtgtcaatacttaaatacacttactt
 E  -  -  -  M  S  Q  S  I  L  N  C  V  N  T  -  I  H  L  L
tagctgtaccctccaacatgagagttattcactttggtgctgg
 -  L  Y  P  P  T  -  E  L  F  T  L  V  L
```

5'3' Frame 2

```
caggttcatcaaaatgtgtgtgttctgtgattgatcttttacttgatgactttgtcgagat
  R  F  I  K  M  C  V  F  C  D  -  S  F  T  -  -  L  C  R  D
aataaagtcacaagatttgtcagtgatttcaaaagtggtcaaggttacaattgactatgc
 N  K  V  T  R  F  V  S  D  F  K  S  G  Q  G  Y  N  -  L  C
tgaaatttcattcatgctttggtgtaaggatggacatgttgaaaccttctacccaaaact
 -  N  F  I  H  A  L  V  -  G  W  T  C  -  N  L  L  P  K  T
acaagcaagtcaagcgtggcaaccaggtgttgcgatgcctaacttgtacaagatgcaaag
 T  S  K  S  S  V  A  T  R  C  C  D  A  -  L  V  Q  D  A  K
aatgcttcttgaaaagtgtgaccttcagaattatggtgaaaatgctgttataccaaaagg
 N  A  S  -  K  V  -  P  S  E  L  W  -  K  C  C  Y  T  K  R
aataatgatgaatgtcgcaaagtatactcaactgtgtcaatacttaaatacacttacttt
 N  N  D  E  C  R  K  V  Y  S  T  V  S  I  L  K  Y  T  Y  F
agctgtaccctccaacatgagagttattcactttggtgctgg
 S  C  T  L  Q  H  E  S  Y  S  L  W  C  W
```

5'3' Frame 3

```
caggttcatcaaaatgtgtgtgttctgtgattgatcttttacttgatgactttgtcgagata
   G  S  S  K  C  V  C  S  V  I  D  L  L  L  D  D  F  V  E  I
ataaagtcacaagatttgtcagtgatttcaaaagtggtcaaggttacaattgactatgct
 I  K  S  Q  D  L  S  V  I  S  K  V  V  K  V  T  I  D  Y  A
gaaatttcattcatgctttggtgtaaggatggacatgttgaaaccttctacccaaaacta
 E  I  S  F  M  L  W  C  K  D  G  H  V  E  T  F  Y  P  K  L
caagcaagtcaagcgtggcaaccaggtgttgcgatgcctaacttgtacaagatgcaaaga
```

Q A S Q A W Q P G V A M P N L Y K M Q R
atgcttcttgaaaagtgtgaccttcagaattatggtgaaaatgctgttataccaaagga
M L L E K C D L Q N Y G E N A V I P K G
ataatgatgaatgtcgcaaagtatactcaactgtgtcaatacttaaatacacttactta
I M M N V A K Y T Q L C Q Y L N T L T L
gctgtaccctccaacatgagagttattcactttggtgctgg
A V P S N M R V I H F G A

3'5' Frame 1

ccagcaccaaagtgaataactctcatgttggagggtacagctaaagtaagtgtatttaag
P A P K - I T L M L E G T A K V S V F K
tattgacacagttgagtatactttgcgacattcatcattattccttttggtataacagca
Y - H S - V Y F A T F I I I P F G I T A
ttttcaccataattctgaaggtcacacttttcaagaagcattctttgcatcttgtacaag
F S P - F - R S H F S R S I L C I L Y K
ttaggcatcgcaacacctggttgccacgcttgacttgcttgtagttttgggtagaaggtt
L G I A T P G C H A - L A C S F G - K V
tcaacatgtccatccttacaccaaagcatgaatgaaatttcagcatagtcaattgtaacc
S T C P S L H Q S M N E I S A - S I V T
ttgaccacttttgaaatcactgacaaatcttgtgactttattatctcgacaaagtcatca
L T T F E I T D K S C D F I I S T K S S
agtaaaagatcaatcacagaacacacacattttgatgaacctg
S K R S I T E H T H F D E P

3'5' Frame 2

ccagcaccaaagtgaataactctcatgttggagggtacagctaaagtaagtgtatttaagt
Q H Q S E - L S C W R V Q L K - V Y L S
attgacacagttgagtatactttgcgacattcatcattattccttttggtataacagcat
I D T V E Y T L R H S S L F L L V - Q H
tttcaccataattctgaaggtcacacttttcaagaagcattctttgcatcttgtacaagt
F H H N S E G H T F Q E A F F A S C T S
taggcatcgcaacacctggttgccacgcttgacttgcttgtagttttgggtagaaggttt
- A S Q H L V A T L D L L V V L G R R F
caacatgtccatccttacaccaaagcatgaatgaaatttcagcatagtcaattgtaacct
Q H V H P Y T K A - M K F Q H S Q L - P
tgaccacttttgaaatcactgacaaatcttgtgactttattatctcgacaaagtcatcaa
- P L L K S L T N L V T L L S R Q S H Q
gtaaaagatcaatcacagaacacacacattttgatgaacctg
V K D Q S Q N T H I L M N L

3'5' Frame 3

ccagcaccaaagtgaataactctcatgttggagggtacagctaaagtaagtgtatttaagta
S T K V N N S H V G G Y S - S K C I - V
ttgacacagttgagtatactttgcgacattcatcattattccttttggtataacagcatt
L T Q L S I L C D I H H Y S F W Y N S I
ttcaccataattctgaaggtcacactttcaagaagcattctttgcatcttgtacaagtt
F T I I L K V T L F K K H S L H L V Q V
aggcatcgcaacacctggttgccacgcttgacttgcttgtagttttgggtagaaggtttc
R H R N T W L P R L T C L - F W V E G F
aacatgtccatccttacaccaaagcatgaatgaaatttcagcatagtcaattgtaacctt
N M S I L T P K H E - N F S I V N C N L
gaccactttgaaatcactgacaaatcttgtgactttattatctcgacaaagtcatcaag
D H F - N H - Q I L - L Y Y L D K V I K
taaaagatcaatcacagaacacacacattttgatgaacctg
- K I N H R T H T F - - T

FIGURE 132

5'3' Frame 1

taggtttttacctacccaggaaaagccaaccaacctcgatctcttgtagatctgttctct
- V F T Y P G K A N Q P R S L V D L F S
aaacgaacaaattaaaatgtctgataatggaccccaatcaaaccaacgtagtgcccccg
K R T N - N V - - W T P I K P T - C P P
cattacatttggtggacccacagattcaactgacaataaccagaatggaggactgcaatg
H Y I W W T H R F N - Q - P E W R T A M
gggcaaggccaaaacagcgccgaccccaaggtttacccaataatattgcgtcttggttca
G Q G Q N S A D P K V Y P I I L R L G S
cagctctcactcagcatggcaaggaggaacttagattccctcgaggccagggcgttccaa
Q L S L S M A R R N L D S L E A R A F Q
tcaacaccaatagtggtccagatgaccaaattggctactaccgaagagctacccgacgag
S T P I V V Q M T K L A T T E E L P D E
ttcgtggtggtgacggcaaaatgaaagagctcagccccagatggtacttctattacctag
F V V V T A K - K S S A P D G T S I T -
gaactggcccagaagcttcacttccctacggcgctaacaaagaaggcatcgtatgggttg
E L A Q K L H F P T A L T K K A S Y G L
caactgagggagccttgaatacacccaaagaccacattggcacccgcaatcctaataaca
Q L R E P - I H P K T T L A P A I L I T
atgctgccaccgtgctacaacttcctcaaggaacaacattgccaaaaggcttctacgcag
M L P P C Y N F L K E Q H C Q K A S T Q
agggaagcagaggcggcagtcaagcctcttctcgctcctcatcacgtagtcgcggtaatt
R E A E A A V K P L L A P H H V V A V I
caagaaattcaactcctggcagcagtaggggaaattctcctgctcgaatggctagcggag
Q E I Q L L A A V G E I L L L E W L A E

```
gtggtgaaactgccctcgcgctattgctgctagacagattgaaccagcttgagagcaaag
 V  V  K  L  P  S  R  Y  C  C  -  T  D  -  T  S  L  R  A  K
tttctggtaaaggccaacaacaacaaggccaaactgtcactaagaaatctgctgctgagg
 F  L  V  K  A  N  N  N  K  A  K  L  S  L  R  N  L  L  L  R
catctaaaaagcctcgccaaaaacgtactgccacaaaacagtacaacgtcactcaagcat
 H  L  K  S  L  A  K  N  V  L  P  Q  N  S  T  T  S  L  K  H
ttgggagacgtggtccagaacaaacccaaggaaatttcggggaccaagacctaatcagac
 L  G  D  V  V  Q  N  K  P  K  E  I  S  G  T  K  T  -  S  D
aaggaactgattacaaacattgggccgcaaattgcacaatttgcct
 K  E  L  I  T  N  I  G  P  Q  I  A  Q  F  A
```

5'3' Frame 2

```
taggtttttacctacccaggaaaagccaaccaacctcgatctcttgtagatctgttctcta
  R  F  L  P  T  Q  E  K  P  T  N  L  D  L  L  -  I  C  S  L
aacgaacaaattaaaatgtctgataatggaccccaatcaaaccaacgtagtgccccccgc
 N  E  Q  I  K  M  S  D  N  G  P  Q  S  N  Q  R  S  A  P  R
attacatttggtggacccacagattcaactgacaataaccagaatggaggactgcaatgg
 I  T  F  G  G  P  T  D  S  T  D  N  N  Q  N  G  G  L  Q  W
ggcaaggccaaaacagcgccgaccccaaggtttacccaataatattgcgtcttggttcac
 G  K  A  K  T  A  P  T  P  R  F  T  Q  -  Y  C  V  L  V  H
agctctcactcagcatggcaaggaggaacttagattccctcgaggccagggcgttccaat
 S  S  H  S  A  W  Q  G  G  T  -  I  P  S  R  P  G  R  S  N
caacaccaatagtggtccagatgaccaaattggctactaccgaagagctacccgacgagt
 Q  H  Q  -  W  S  R  -  P  N  W  L  L  P  K  S  Y  P  T  S
tcgtggtggtgacggcaaaatgaaagagctcagccccagatggtacttctattacctagg
 S  W  W  -  R  Q  N  E  R  A  Q  P  Q  M  V  L  L  L  P  R
aactggcccagaagcttcacttccctacggcgctaacaaagaaggcatcgtatgggttgc
 N  W  P  R  S  F  T  S  L  R  R  -  Q  R  R  H  R  M  G  C
aactgagggagccttgaatacacccaaagaccacattggcacccgcaatcctaataacaa
 N  -  G  S  L  E  Y  T  Q  R  P  H  W  H  P  Q  S  -  -  Q
tgctgccaccgtgctacaacttcctcaaggaacaacattgccaaaaggcttctacgcaga
 C  C  H  R  A  T  T  S  S  R  N  N  I  A  K  R  L  L  R  R
gggaagcagaggcggcagtcaagcctcttctcgctcctcatcacgtagtcgcggtaattc
 G  K  Q  R  R  Q  S  S  L  F  S  L  L  I  T  -  S  R  -  F
aagaaattcaactcctggcagcagtaggggaaattctcctgctcgaatggctagcggagg
 K  K  F  N  S  W  Q  Q  -  G  K  F  S  C  S  N  G  -  R  R
tggtgaaactgccctcgcgctattgctgctagacagattgaaccagcttgagagcaaagt
 W  -  N  C  P  R  A  I  A  A  R  Q  I  E  P  A  -  E  Q  S
ttctggtaaaggccaacaacaacaaggccaaactgtcactaagaaatctgctgctgaggc
 F  W  -  R  P  T  T  T  R  P  N  C  H  -  E  I  C  C  -  G
atctaaaaagcctcgccaaaaacgtactgccacaaaacagtacaacgtcactcaagcatt
 I  -  K  A  S  P  K  T  Y  C  H  K  T  V  Q  R  H  S  S  I
tgggagacgtggtccagaacaaacccaaggaaatttcggggaccaagacctaatcagaca
 W  E  T  W  S  R  T  N  P  R  K  F  R  G  P  R  P  N  Q  T
aggaactgattacaaacattgggccgcaaattgcacaatttgcct
 R  N  -  L  Q  T  L  G  R  K  L  H  N  L  P
```

5'3' Frame 3

```
taggtttttacctacccaggaaaagccaaccaacctcgatctcttgtagatctgttctctaa
   G  F  Y  L  P  R  K  S  Q  P  T  S  I  S  C  R  S  V  L  -
acgaacaaattaaaatgtctgataatggaccccaatcaaaccaacgtagtgcccccgca
 T  N  K  L  K  C  L  I  M  D  P  N  Q  T  N  V  V  P  P  A
ttacatttggtggacccacagattcaactgacaataaccagaatggaggactgcaatggg
 L  H  L  V  D  P  Q  I  Q  L  T  I  T  R  M  E  D  C  N  G
gcaaggccaaaacagcgccgaccccaaggtttacccaataatattgcgtcttggttcaca
 A  R  P  K  Q  R  R  P  Q  G  L  P  N  N  I  A  S  W  F  T
gctctcactcagcatggcaaggaggaacttagattccctcgaggccagggcgttccaatc
 A  L  T  Q  H  G  K  E  E  L  R  F  P  R  G  Q  G  V  P  I
aacaccaatagtggtccagatgaccaaattggctactaccgaagagctacccgacgagtt
 N  T  N  S  G  P  D  D  Q  I  G  Y  Y  R  R  A  T  R  R  V
cgtggtggtgacggcaaaatgaaagagctcagccccagatggtacttctattacctagga
 R  G  G  D  G  K  M  K  E  L  S  P  R  W  Y  F  Y  Y  L  G
actggcccagaagcttcacttccctacggcgctaacaaagaaggcatcgtatgggttgca
 T  G  P  E  A  S  L  P  Y  G  A  N  K  E  G  I  V  W  V  A
actgagggagccttgaatacacccaaagaccacattggcacccgcaatcctaataacaat
 T  E  G  A  L  N  T  P  K  D  H  I  G  T  R  N  P  N  N  N
gctgccaccgtgctacaacttcctcaaggaacaacattgccaaaaggcttctacgcagag
 A  A  T  V  L  Q  L  P  Q  G  T  T  L  P  K  G  F  Y  A  E
ggaagcagaggcggcagtcaagcctcttctcgctcctcatcacgtagtcgcggtaattca
 G  S  R  G  G  S  Q  A  S  S  R  S  S  S  R  S  R  G  N  S
agaaattcaactcctggcagcagtaggggaaattctcctgctcgaatggctagcggaggt
 R  N  S  T  P  G  S  S  R  G  N  S  P  A  R  M  A  S  G  G
ggtgaaactgccctcgcgctattgctgctagacagattgaaccagcttgagagcaaagtt
 G  E  T  A  L  A  L  L  L  L  D  R  L  N  Q  L  E  S  K  V
tctggtaaaggccaacaacaacaaggccaaactgtcactaagaaatctgctgctgaggca
 S  G  K  G  Q  Q  Q  Q  G  Q  T  V  T  K  K  S  A  A  E  A
tctaaaaagcctcgccaaaaacgtactgccacaaaacagtacaacgtcactcaagcattt
 S  K  K  P  R  Q  K  R  T  A  T  K  Q  Y  N  V  T  Q  A  F
gggagacgtggtccagaacaaacccaaggaaatttcggggaccaagacctaatcagacaa
 G  R  R  G  P  E  Q  T  Q  G  N  F  G  D  Q  D  L  I  R  Q
ggaactgattacaaacattgggccgcaaattgcacaatttgcct
 G  T  D  Y  K  H  W  A  A  N  C  T  I  C
```

3'5' Frame 1

```
aggcaaattgtgcaatttgcggcccaatgtttgtaatcagttccttgtctgattaggtct
 R  Q  I  V  Q  F  A  A  Q  C  L  -  S  V  P  C  L  I  R  S
tggtccccgaaatttccttgggtttgttctggaccacgtctcccaaatgcttgagtgacg
 W  S  P  K  F  P  W  V  C  S  G  P  R  L  P  N  A  -  V  T
ttgtactgttttgtggcagtacgtttttggcgaggctttttagatgcctcagcagcagat
```

L Y C F V A V R F W R G F L D A S A A D
ttcttagtgacagtttggccttgttgttgttggcctttaccagaaactttgctctcaagc
F L V T V W P C C C W P L P E T L L S S
tggttcaatctgtctagcagcaatagcgcgagggcagtttcaccacctccgctagccatt
W F N L S S S N S A R A V S P P P L A I
cgagcaggagaatttcccctactgctgccaggagttgaatttcttgaattaccgcgacta
R A G E F P L L L P G V E F L E L P R L
cgtgatgaggagcgagaagaggcttgactgccgcctctgcttccctctgcgtagaagcct
R D E E R E E A - L P P L L P S A - K P
tttggcaatgttgttccttgaggaagttgtagcacggtggcagcattgttattaggattg
F G N V V P - G S C S T V A A L L L G L
cgggtgccaatgtggtctttgggtgtattcaaggctccctcagttgcaacccatacgatg
R V P M W S L G V F K A P S V A T H T M
ccttctttgttagcgccgtagggaagtgaagcttctgggccagttcctaggtaatagaag
P S L L A P - G S E A S G P V P R - - K
taccatctggggctgagctctttcatttgccgtcaccaccacgaactcgtcgggtagct
Y H L G L S S F I L P S P P R T R R V A
cttcggtagtagccaatttggtcatctggaccactattggtgttgattggaacgccctgg
L R - - P I W S S G P L L V L I G T P W
cctcgagggaatctaagttcctccttgccatgctgagtgagagctgtgaaccaagacgca
P R G N L S S S L P C - V R A V N Q D A
atattattgggtaaaccttggggtcggcgctgttttggccttgccccattgcagtcctcc
I L L G K P W G R R C F G L A P L Q S S
attctggttattgtcagttgaatctgtgggtccaccaaatgtaatgcggggggcactacg
I L V I V S - I C G S T K C N A G G T T
ttggtttgattggggtccattatcagacattttaatttgttcgtttagagaacagatcta
L V - L G S I I R H F N L F V - R T D L
caagagatcgaggttggttggcttttcctgggtaggtaaaaaccta
Q E I E V G W L F L G R - K P

3'5' Frame 2

aggcaaattgtgcaatttgcggcccaatgtttgtaatcagttccttgtctgattaggtctt
G K L C N L R P N V C N Q F L V - L G L
ggtccccgaaatttccttgggtttgttctggaccacgtctcccaaatgcttgagtgacgt
G P R N F L G F V L D H V S Q M L E - R
tgtactgttttgtggcagtacgttttggcgaggctttttagatgcctcagcagcagatt
C T V L W Q Y V F G E A F - M P Q Q Q I
tcttagtgacagtttggccttgttgttgttggcctttaccagaaactttgctctcaagct
S - - Q F G L V V V G L Y Q K L C S Q A
ggttcaatctgtctagcagcaatagcgcgagggcagtttcaccacctccgctagccattc
G S I C L A A I A R G Q F H H L R - P F
gagcaggagaatttcccctactgctgccaggagttgaatttcttgaattaccgcgactac
E Q E N F P Y C C Q E L N F L N Y R D Y
gtgatgaggagcgagaagaggcttgactgccgcctctgcttccctctgcgtagaagcctt
V M R S E K R L D C R L C F P L R R S L
ttggcaatgttgttccttgaggaagttgtagcacggtggcagcattgttattaggattgc

L A M L F L E E V V A R W Q H C Y - D C
gggtgccaatgtggtctttgggtgtattcaaggctccctcagttgcaacccatacgatgc
G C Q C G L W V Y S R L P Q L Q P I R C
cttctttgttagcgccgtagggaagtgaagcttctgggccagttcctaggtaatagaagt
L L C - R R R E V K L L G Q F L G N R S
accatctggggctgagctctttcatttgccgtcaccaccacgaactcgtcgggtagctc
T I W G - A L S F C R H H H E L V G - L
ttcggtagtagccaatttggtcatctggaccactattggtgttgattggaacgccctggc
F G S S Q F G H L D H Y W C - L E R P G
ctcgagggaatctaagttcctccttgccatgctgagtgagagctgtgaaccaagacgcaa
L E G I - V P P C H A E - E L - T K T Q
tattattgggtaaaccttggggtcggcgctgttttggccttgccccattgcagtcctcca
Y Y W V N L G V G A V L A L P H C S P P
ttctggttattgtcagttgaatctgtgggtccaccaaatgtaatgcggggggcactacgt
F W L L S V E S V G P P N V M R G A L R
tggtttgattggggtccattatcagacattttaatttgttcgtttagagaacagatctac
W F D W G P L S D I L I C S F R E Q I Y
aagagatcgaggttggttggcttttcctgggtaggtaaaaaccta
K R S R L V G F S W V G K N L

3'5' Frame 3

aggcaaattgtgcaatttgcggcccaatgtttgtaatcagttccttgtctgattaggtcttg
A N C A I C G P M F V I S S L S D - V L
gtccccgaaatttccttgggtttgttctggaccacgtctcccaaatgcttgagtgacgtt
V P E I S L G L F W T T S P K C L S D V
gtactgttttgtggcagtacgtttttggcgaggcttttagatgcctcagcagcagattt
V L F C G S T F L A R L F R C L S S R F
cttagtgacagtttggccttgttgttgttggcctttaccagaaactttgctctcaagctg
L S D S L A L L L L A F T R N F A L K L
gttcaatctgtctagcagcaatagcgcgagggcagtttcaccacctccgctagccattcg
V Q S V - Q Q - R E G S F T T S A S H S
agcaggagaatttcccctactgctgccaggagttgaatttcttgaattaccgcgactacg
S R R I S P T A A R S - I S - I T A T T
tgatgaggagcgagaagaggcttgactgccgcctctgcttccctctgcgtagaagccttt
- - G A R R G L T A A S A S L C V E A F
tggcaatgttgttccttgaggaagttgtagcacggtggcagcattgttattaggattgcg
W Q C C S L R K L - H G G S I V I R I A
ggtgccaatgtggtctttgggtgtattcaaggctccctcagttgcaacccatacgatgcc
G A N V V F G C I Q G S L S C N P Y D A
ttctttgttagcgccgtagggaagtgaagcttctgggccagttcctaggtaatagaagta
F F V S A V G K - S F W A S S - V I E V
ccatctggggctgagctctttcatttgccgtcaccaccacgaactcgtcgggtagctct
P S G A E L F H F A V T T T N S S G S S
tcggtagtagccaatttggtcatctggaccactattggtgttgattggaacgccctggcc
S V V A N L V I W T T I G V D W N A L A
tcgagggaatctaagttcctccttgccatgctgagtgagagctgtgaaccaagacgcaat

S R E S K F L L A M L S E S C E P R R N
attattgggtaaaccttggggtcggcgctgttttggccttgccccattgcagtcctccat
I I G - T L G S A L F W P C P I A V L H
tctggttattgtcagttgaatctgtgggtccaccaaatgtaatgcgggggcactacgtt
S G Y C Q L N L W V H Q M - C G G H Y V
ggtttgattggggtccattatcagacattttaatttgttcgtttagagaacagatctaca
G L I G V H Y Q T F - F V R L E N R S T
agagatcgaggttggttggcttttcctgggtaggtaaaaaccta
R D R G W L A F P G - V K T

FIGURE 133

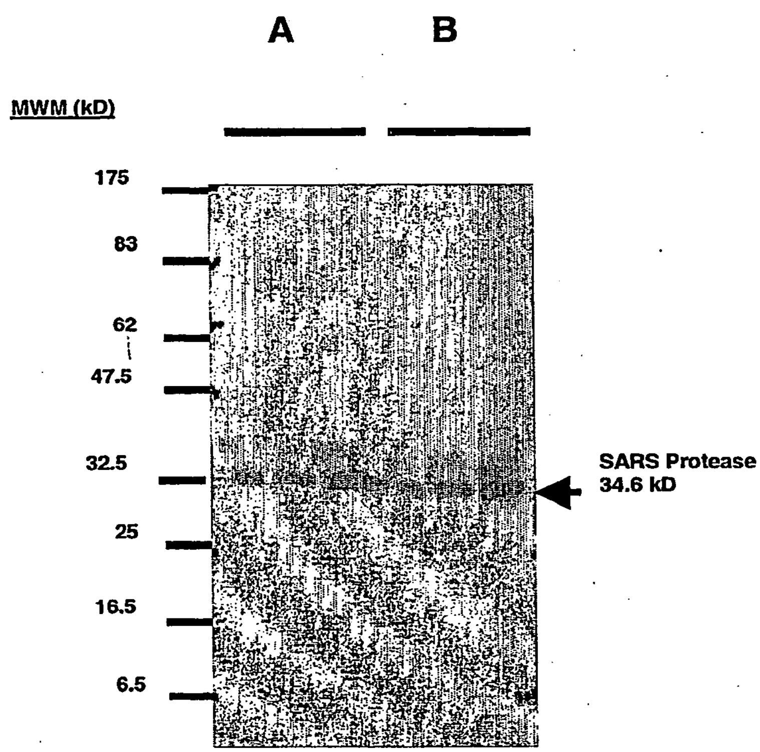
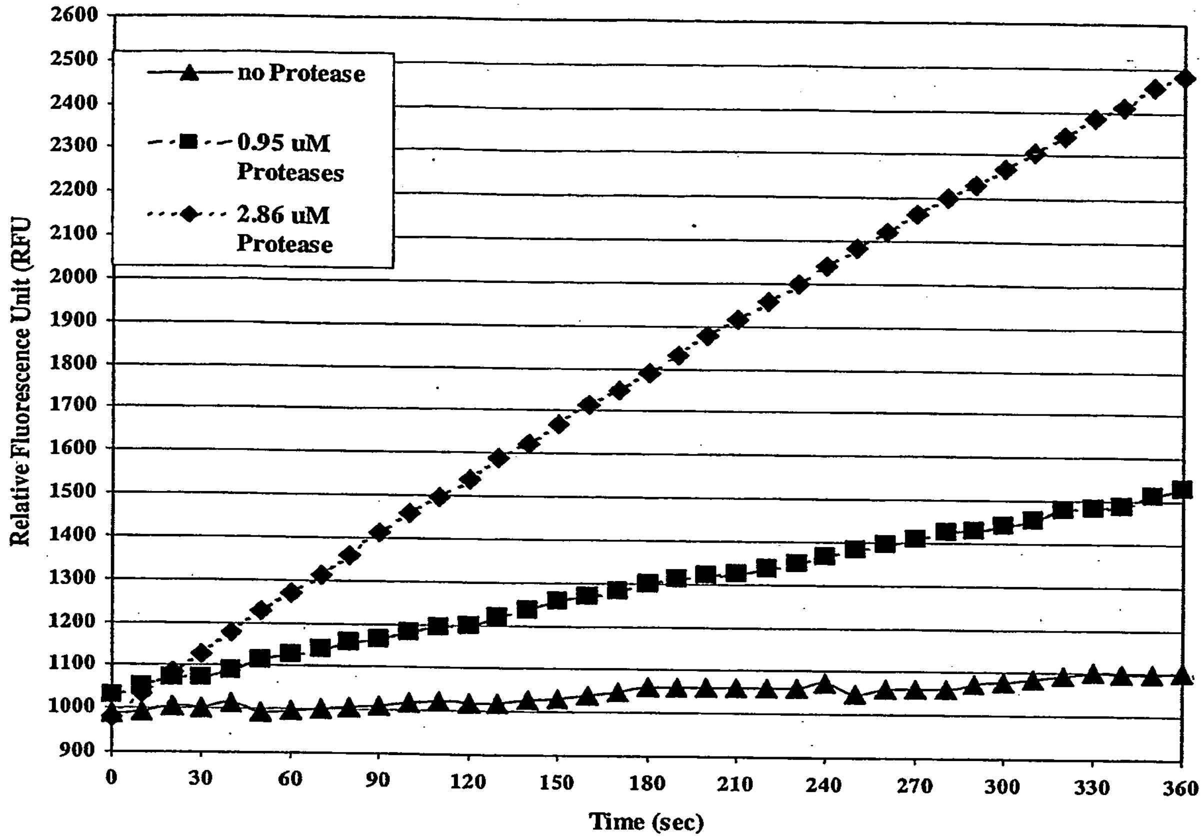


FIGURE 134


no Protease
0.95 uM Proteases
2.86 uM Protease
Relative Fluorescence Unit (RFU)
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
0
30
60
90
120
150
180
210
240
270
300
330
360
Time (sec)

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[Continued on next page]

(54) Title: THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS

(57) Abstract: An outbreak of a virulent respiratory virus, now known as Severe Acute Respiratory Syndrome (SARS), was identified in Hong Kong, China and a growing number of countries around the world in 2003. The invention relates to nucleic acids and proteins from the SARS coronavirus. These nucleic acids and proteins can be used in the preparation and manufacture of vaccine formulations, diagnostic reagents, kits, etc. The invention also provides methods for treating SARS by administering small molecule antiviral compounds, as well as methods of identifying potent small molecules for the treatment of SARS.

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEIRIS J S M ET AL: "Coronavirus as a possible cause of severe acute respiratory syndrome." LANCET. 19 APR 2003, vol. 361, no. 9366, 8 April 2003 (2003-04-08), pages 1319-1325, XP004421148 ISSN: 0140-6736 cited in the application published online on 8 April 2003 (http://image.thelancet.com/extras/03art3477web.pdf) page 1320, left-hand column - page 1321, right-hand column page 1322, right-hand column page 1322, left-hand column; figure 3	1-28, 73-77, 79,83, 85-109, 114-120

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2005	08. 06. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Renggli-Zulliger, N

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DROSTEN CHRISTIAN ET AL: "Identification of a novel coronavirus in patients with severe acute respiratory syndrome." NEW ENGLAND JOURNAL OF MEDICINE, vol. 348, no. 20, 10 April 2003 (2003-04-10), pages 1967-1976, XP002305233 ISSN: 0028-4793 cited in the application published online on 10 April 2003 (http://www.nejm.org) table 1, Figure 1figure 1; table 1	1-28, 73-77, 79,83, 85-109, 114-120
X	-& WORLD HEALTH ORGANIZATION, 'Online! 25 March 2003 (2003-03-25), XP002319429 Retrieved from the Internet: URL:(http://www.who.int/csr/sars/primers/en)> 'retrieved on 2005-02-10! BNI-1 primers were published online on 24 and 25 March 2003 according to Drosten et al. the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
P,X	DATABASE GENBANK 'Online! NATIONAL CENTER FOR BIO; 14 April 2003 (2003-04-14), BCCA GENOME SCIENCES CENTER BRITISH COLUMBIA: "SARS coronavirus Tor2" XP002319430 retrieved from NCBI Database accession no. AY274119 page 1971, left-hand column; figure 1; table 1	1-28, 73-77, 79,83, 85-109, 114-120
P,X	-& MARRA M A ET AL: "The genome sequence of the SARS-associated coronavirus" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 300, no. 5624, 30 May 2003 (2003-05-30), pages 1399-1404, XP002269483 ISSN: 0036-8075 Published online 1 may 2003 Tables 1-3 -----	1-28, 73-77, 79,83, 85, 98-109, 114-120
P,X	ROTA PAUL A ET AL: "Characterization of a novel coronavirus associated with severe acute respiratory syndrome" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 300, no. 5624, 30 May 2003 (2003-05-30), pages 1394-1399, XP002288938 ISSN: 0036-8075 tables 1-3 ----- -/--	1-28, 73-77, 79,83, 85-109, 114-120

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2004/091524 A (ACAMBIS INC; MONATH, THOMAS, P; KLEANTHOUS, HAROLD) 28 October 2004 (2004-10-28) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
E	WO 2004/085650 A (THE UNIVERSITY OF HONG KONG) 7 October 2004 (2004-10-07) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
E	WO 2004/085633 A (THE UNIVERSITY OF HONG KONG) 7 October 2004 (2004-10-07) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
E	WO 2004/085455 A (THE UNIVERSITY OF HONG KONG) 7 October 2004 (2004-10-07) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
E	WO 2004/096842 A (BC CANCER AGENCY; PLUMMER, FRANK; FELDMANN, HEINZ; JONES, STEVEN; LI,) 11 November 2004 (2004-11-11) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120
E	WO 2004/092208 A (HER MAJESTY THE QUEEN IN RIGHT OF CANADA AS REPRESENTED BY THE MINISTE) 28 October 2004 (2004-10-28) the whole document -----	1-28, 73-77, 79,83, 85-109, 114-120

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/011710

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004091524 A	28-10-2004	WO 2004091524 A2	28-10-2004
WO 2004085650 A	07-10-2004	WO 2004085650 A1	07-10-2004
		WO 2004085455 A1	07-10-2004
		WO 2004085633 A1	07-10-2004
		US 2005009009 A1	13-01-2005
WO 2004085633 A	07-10-2004	WO 2004085650 A1	07-10-2004
		WO 2004085455 A1	07-10-2004
		WO 2004085633 A1	07-10-2004
		US 2005009009 A1	13-01-2005
WO 2004085455 A	07-10-2004	WO 2004085650 A1	07-10-2004
		WO 2004085455 A1	07-10-2004
		WO 2004085633 A1	07-10-2004
		US 2005009009 A1	13-01-2005
WO 2004096842 A	11-11-2004	WO 2004096842 A2	11-11-2004
WO 2004092208 A	28-10-2004	WO 2004092208 A2	28-10-2004

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/011710

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 77-83, 117-120 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-12(partially), 13(partially), 14, 15, 16-21(partially), 22-28(partially) 73-77 (partially), 79(partially), 83(partially), 85-93(partially), 94-98 99-104(partially), 105, 106-107(partially), 108, 109, 114-120(partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1

claims: 1-12(partially),13(partially),14, 15, 16-21(partially), 22-28(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially),94-98, 99-104(partially),105, 106-107(partially), 108, 109, 114-120(partially)
The polypeptide is a spike (S) polypeptide encoded by SEQ. ID n 6042, nucleic acid encoding the spike protein, fragments thereof, antibodies specific for the spike protein, immunoassays using these antibodies, a vaccine comprising a spike protein, a viral vector comprising the protein, an immunogenic fragment thereof, double stranded RNA thereof, the recombinaint expression thereof and the medical use thereof.

Invention 2

claims:1-12(partially),13(partially),16-21(partially), 22-28(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
the polypeptide is an envelope (E) polypeptide encoded by SEQ. ID n 6045, nucleic acid encoding the E protein, fragments thereof, antibodies specific for the E protein, immunoassays using that antibody, a vaccine comprising an E protein, a viral vector comprising the protein,double stranded RNA thereof the recombinant expression thereof and the medical use thereof.

Invention 3

claims :1-12(partially),13(partially),16-21(partially), 22-28(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
the polypeptide is a membrane (M) polypeptide encoded by SEQ. ID n 6046, nucleic acid encoding the M protein, fragment thereof, antibodies specific for the M protein, immunoassays using that antibody, a vaccine comprising a M protein, a viral vector comprising the protein, double stranded RNA thereof, the recombinant expression thereof and the medical use thereof.

Invention 4

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims :1-12(partially),13(partially),16-21(partially), 22-27(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
the polypeptide is a hemagglutinin esterase (HE) polypeptide, nucleic acid encoding the HE protein, fragment thereof, antibodies specific for the HE protein, immunoassays using that antibody, a vaccine comprising a HE protein, a viral vector comprising the protein, double stranded RNA thereof, the recombinant expression thereof and the medical use thereof.

Invention 5

claims:1-12(partially),13(partially),16-21(partially), 22-28(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
The polypeptide is a nucleocapsid (N) polypeptide encoded by SEQ. ID n 6052, nucleic acid encoding the N protein, fragment thereof, antibodies specific for the N protein, immunoassays using that antibody, a vaccine comprising a N protein, a viral vector comprising the protein, double stranded RNA thereof, the recombinant expression thereof and the medical use thereof.

Invention 6

claims:1-12(partially),13(partially),16-21(partially), 22-27(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
The ORF1a polypeptide encoded by SEQ. ID n 6039, the preoteolytic fragments thereof such as NSP1-Nsp-7 corresponding to SEQ.ID n 9766-9774),nucleic acid encoding these proteins, fragments thereof, antibodies specific for these proteins, immunoassays using these antibodies, a vaccine comprising the protein, a viral vector comprising the protein,double stranded RNA thereof, the recombinant expression thereof and the medical use thereof.

Invention 7

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

claims:1-12(partially),13(partially),16-21(partially), 22-27(partially), 73-77(partially), 79(partially), 83(partially), 85-93(partially), 99-104(partially), 106-107(partially), 114-120(partially)
The ORF1ab polypeptide encoded by SEQ. ID n 6041, the preoteolytic fragments thereof such as NSP9-Nsp-13 corresponding to SEQ.ID n 9775-9779),nucleic acid encoding these proteins, fragments thereof, antibodies specific for these proteins, immunoassays using these antibodies, a vaccine comprising the protein, a viral vector comprising the protein, double stranded RNA thereof, the recombinant expression thereof and the medical use thereof.

Invention 8

claims: 22(partially), 29-58, 84, 110-113(partially),114-118(partially):
A vaccine comprising an inactivated/attenuated SARS virus, a method of inactivating the SARS virus, a method of making an inactivated SARS vaccine

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Invention 9:

claims: 77(partially), 78, 79(partially),80-82, 83 (patially),110-113(partially), 119-120 (partially)
A method of treatment of a patient suffering from SARS and a method of identifying a therapeutically active agent comprising measuring the attenuation of a SARS related enzyme, a method of treatment using a therapeutical agent of claims 77-82.

Invention 10-7760

claims :59-72(partially)
A single-stranded oligonucleotide selected from the group consisting of the SEQ. IDs 21-6020, 6076-6568, 6586-6587, 7292-7301, 7325-7328, 7332-7352, 7353-7385, 10235-10298, 10352-10504, 10580-11322, 11325-11551 (taken from the list of claim number 59), PCR kit comprising these primers, a method of detecting the presence of SARS virus in a sample using PCR.
